INDICATOR SIGNIFICANCE OF SOME SHRUBS IN THE ESCALANTE DESERT, UTAH[1]

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Introduction

For many years it has been recognized that native plants such as sagebrush (*Artemisia tridentata*), shadscale (*Atriplex confertifolia*), and greasewood (*Sarcobatus vermiculatus*) may serve as important indicators of the agricultural possibilities of new lands. It is generally considered that sagebrush indicates soils well suited to agriculture, while shadscale grows on soils less well suited and greasewood on soils unfavorable for agriculture (**6, 7, 10**); but these generalizations do not always hold. Admittedly, growth of these plants is a measure of the conditions to which they are subjected, but their use as indicators is limited until the factors which control their growth are better understood.

Some progress has been made in describing the salt and alkali tolerance of indicator plants, but quantitative data on the tolerance and relation of these plants to hydrogen-ion activity (usually expressed as pH), total soluble salts, exchangeable sodium, and other soil factors are limited. For example, it is not known whether greasewood (*a*) requires a soil of high pH value and high total salt and exchangeable-sodium content; (*b*) brings about increases in pH, salt, and sodium as a result of its growth in normal or slightly saline-alkali soil; or (*c*) exists in soils high in pH, salt, and sodium because greasewood tolerates these conditions better than other plant species. That is, assuming favorable climatic factors, do the characteristics of the soil determine the character of the vegetation, or do the characteristics of the indicator plants determine in part the chemical and physical nature of the soil in which they grow?

HILGARD (**5, 6**) was one of the first investigators to recognize the importance of plant indicators in the appraisal of the agricultural possibilities of new lands. He was of the opinion that the native plant growth on alkali lands affords a means of obtaining information regarding both the quality and the quantity of the saline constituents of soils and stated that "the most superficial observation shows that certain plants indicate extremely strong alkali lands where they occupy the ground alone; others indicate preeminently the presence of common salt; the presence or absence of still others form definite or probable indications of reclaimability or non-reclaimability." Recently, SAMPSON (**9**) stated: "In the future a broader use of indicator communities and species is likely, but such use is sure to be backed by sounder evidences than it has at this time. . . . There must first be more critical study of the growth requirements of . . . the indicator plant."

In 1911 SHANTZ (**10**) considered natural vegetation a fair indicator of the character of the soil. KEARNEY *et al.* (**7**) and HARRIS *et al.* (**2**), working in the Tooele

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Valley, Utah, made quantitative studies of plant communities as indicators of alkali. The former authors determined moisture equivalent, wilting coefficient, and salt content; the latter attempted to correlate the electrical resistance of the soil paste with the osmotic concentration and chloride content of tissue fluids. HARRIS and LAWRENCE (**3**) studied desert plants in Arizona and found that the osmotic pressure of expressed saps was positively correlated with the salt content of the soil. From studies conducted in Utah, HARRIS and YOUNG (**4**) concluded that "the chemical composition of the soil determines the actual position of the greasewood and sagebrush individuals" and that "it appears that desert plants play a minor role, if any, in bringing about chemical changes in the soil that might influence the type of vegetation a habitat may ultimately carry."

On the other hand, BILLINGS (**1**) has described big greasewood, big greasewood–shadscale, sagebrush, and other plant associations of the Carson desert region in western Nevada. In the big greasewood association (*Sarcobatus vermiculatus*) he found that "the soils seem to be solonchak with rather high alkalinity throughout the profile, ranging from pH 8.5 to about pH 9.5. The soils contain rather high quantities of soluble salts, the percentage increasing from the lower part of the profile to the upper." During a study of soils from typical plant communities in six counties in Utah, FLOWERS[4] obtained evidence that values for pH and soluble salt were higher in soils from shadscale and greasewood communities than from sagebrush communities. Also, ROBERTS (**8**), in connection with soil-survey studies in the western states, concluded that "shrubs such as greasewood and shadscale are directly responsible for significant changes in some of the chemical characteristics of the soil profile."

During the course of field trips in Arizona, Colorado, Idaho, Nevada, New Mexico, and Utah in the years 1947–1951, numerous paired soil samples were taken from under various desert shrubs and from adjacent barren[5] soil, and pH values were determined. In the case of some plant species no significant differences were found, while in others the pH values of soils under the shrubs were strikingly different from those of intervening barren soil. Therefore, a systematic study was initiated in 1949 and continued in 1950 with the following objectives: (*a*) to ascertain the relation of vigor, age, and distribution of indicator plants to the physical and chemical characteristics of the soils of their habitats; (*b*) to compare the soils occupied by the root systems of indicator plants with the soils in the adjacent interspaces; and (*c*) to determine the relation between the chemical composition of plant parts and differences in soil characteristics. No attempt was made to establish either the mechanism responsible for the observed changes in soil characteristics or the length of time required for these changes to take place.

The results presented in this paper were obtained principally on sagebrush (*Artemisia tridentata*), shadscale (*Atriplex confertifolia*), and greasewood (*Sarcobatus vermiculatus*) growing in the portion of the Escalante Desert, Utah, known as the Beryl-Enterprise area. A few scattered observations were made

[4] From an unpublished report of Seville Flowers, University of Utah, presented at Ecological Society of America meetings held in Salt Lake City, June, 1942.

[5] In this paper soil referred to as "barren" is shrubless but may carry a growth of annual plants in the spring.

here and elsewhere on rabbitbrush (*Chrysothamnus* spp.), pickleweed (*Allenrolfea occidentalis*), salt grass (*Distichlis stricta*), gray molly (*Kochia vestita*), fourwing saltbush (*Atriplex canescens*), creosote bush (*Larrea divaricata*), and Australian saltbush (*Atriplex semibaccata*).

If conditions are favorable, sagebrush possesses a generalized root system with a highly developed system of laterals in the upper soil and a deeply penetrating taproot. Shadscale generally has widespreading shallow lateral roots and a thick vertical taproot which may reach a depth of 4–8 feet. Greasewood has a moderate amount of relatively coarse shallow roots, which may extend to a depth of 2–3 feet, and a strong taproot which may penetrate to a depth of 15 feet or more. STEWART *et al.* (**12**), in a study of the effect of unrestricted grazing, have given good descriptions of the climate, plant associations, and rooting habits of plants of the Beryl-Enterprise area. SHANTZ and PIEMEISEL (**11**) studied the types of vegetation in the Escalante Valley, Utah, as related to environmental conditions including physiography, climate, and salinity of soils. They considered plant communites on nonsaline and saline soils and discussed the plant associations under each category. Those occurring on saline soils were the shadscale, greasewood, saltsage, saltgrass, and pickleweed associations. In each instance the authors reported soil-moisture conditions and salt content to a depth of 4 feet and related these factors to the botanical composition and distribution of dominant species or indicator plants in typical areas in the Valley.

This area comprises about 600 square miles in southwestern Utah. It is oval in shape and is essentially a basin surrounded by dissected hills and mountain ranges. Elevations range from about 5100 feet above sea level in the lowest part of the basin to about 5500 feet where the alluvial fans merge with the rugged mountain slopes. The soils are derived from various rocks such as rhyolite, andesite, basalt, and obsidian and from sedimentary formations. The soils of the lower portion of the basin, including about half the area, apparently have been affected by a lake or ground water. Salts accumulated in or near the surface soil, and this resulted in the development of extensive areas of saline and saline-alkali soils.[6] As the water level receded, many of the saline soils were leached free of soluble salts but not of exchangeable sodium, thus accounting for the development of areas of relatively nonsaline alkali soils. Differential weathering, flooding, leaching, and variable ground-water conditions have given rise to an extremely complex pattern of soils.

The climate of the area is typical of the drier portions of the northern desert. Rainfall averages less than 10 inches per

[6] Alkali, saline-alkali, and saline soils contain sufficient exchangeable sodium or soluble salts or both to interfere with the growth of most plants. They are defined as follows: *Alkali soil:* A soil in which the exchangeable-sodium percentage is generally greater than 15, the conductivity of the saturation extract is less than 4 millimhos/cm at 25° C., and the pH of the saturated soil usually exceeds 8.5. *Saline-alkali soil:* A soil in which the exchangeable-sodium percentage is greater than 15, the conductivity of the saturation extract is greater than 4 millimhos/cm at 25° C., and the pH of the saturated soil is frequently less than 8.5. *Saline soil:* A soil in which the conductivity of the saturation extract is greater than 4 millimhos/cm at 25° C., the exchangeable-sodium percentage is less than 15, and the pH of the saturated paste is usually less than 8.5 (**13**). The term "exchangeable-sodium percentage" indicates the degree of saturation of the soil-exchange-complex with sodium and is expressed as a percentage of the cation-exchange capacity (**13**). The conductivity of the saturation extract (specific electrical conductance in millimhos/cm at 25° C.) is a means of expressing the salinity of a soil in terms related to plant growth. Three millimhos/cm are roughly equivalent to 0.1% salt on the dry-soil basis (**13**).

year except close to the mountains and is subject to wide fluctuations; winds are frequent and of fairly high velocity, and so potential evaporation is high, probably more than 60 inches annually. The summers are warm, and the winters are cold, with wide diurnal fluctuations and extremes in temperature ranging from −35° F. in winter to over 100° F. in summer. The average frost-free period is 115 days, usually from mid-May to mid-September.

Results

RELATION OF pH OF SOIL TO DISTRIBUTION OF INDICATOR PLANTS.—A number of paired surface-soil samples[7] were taken at random from under greasewood, shadscale, sagebrush, and other shrubs and from the adjacent barren soil. In addition, short transects were established in pure stands of greasewood and shadscale, and paired soil samples were taken from under each of these plants and from the adjacent interplant surface soil along the transects. The pH values of most of these samples were determined immediately in the field, using thymol blue indicator; many of the samples were taken to the laboratory, and pH values were determined with the glass electrode. In most cases the pH values of both a saturated paste (pH_s) and a 1:10 soil-water suspension (pH_{10}) were determined. The pH of the paste probably is more closely related to plant growth than the pH of more dilute systems. However, the pH of the 1:10 soil-water suspension may have greater diagnostic value because (*a*) the range of pH values obtainable is greater, owing to increased hydrolysis upon dilution, and (*b*) the measurement is more conveniently made in a dilute suspension, especially by workers in the field. Other measurements, such as salt content and exchangeable-sodium content, also were made on these samples.

Most significantly and without exception the surface soil under greasewood had a higher pH than the adjacent barren soil. In most cases the pH was higher under shadscale than in the adjacent soil. On the other hand, there was generally no significant difference in the pH under sagebrush, rabbitbrush, or pickleweed, as compared with adjacent shrubless soil.

The data have been summarized (table 1) by averaging the pH values of the surface-soil samples from under each of the three principal plant species and listing the calculated value along with the average pH of the corresponding adjacent shrubless soil samples. Arithmetic averages were calculated, rather than converting to hydrogen-ion concentration, averaging, and reconverting to pH, since interest is centered in the average differences rather than in hydrogen-ion concentration as such. In this particular area sagebrush plants do not have a significant effect upon soil pH, while shadscale and greasewood produce increasingly large effects. Furthermore, the distribution of these three plant associations is correlated with soil pH; barren soils in the sagebrush areas have the lowest pH, barren soils of the shadscale association are intermediate, and those of the greasewood association are highest.

RELATION OF PLANT SIZE TO pH, SALT, AND EXCHANGEABLE-SODIUM CONTENT OF SOIL.—After it was established that soil pH is affected by the growth of shadscale and greasewood, field procedures were used to investigate these species in relation to the exchangeable-sodium and soluble salt contents of the soil under and adjacent to them.

Pure stands of shadscale and greasewood on the Antelope Springs soil series were investigated. This series consists of

[7] Unless otherwise stated, surface-soil samples generally were taken to a depth of 2 inches.

intrazonal alkali soils that occur on nearly level alluvial plains. In the Beryl-Enterprise area this series is mapped in the Sierozem soil zone. The parent alluvium has been derived from a variety of igneous and sedimentary rocks and is moderately well developed in the surface. A more advanced degree of development is apparent in the reddish-brown color and finer texture of the B horizon, together with its granular to subangular blocky structure. The surface soil to a depth of 1–3 inches is usually platy and vesicular, loam to clay loam in texture, and is very low in organic material. The soils are calcareous throughout. The pH of the surface soil made up to a paste is usually about 8.0 and that of the subsoil is generally 8.5 or above. The salt content is low except in basinlike areas where leaching and drainage may not be good. The vegetative cover consists mainly of greasewood and shadscale.

From each plant association twenty surface-soil samples were taken in pairs from under and adjacent to plants with crowns of selected diameter. Samples from under the plants were grouped and mixed according to species and crown diameter; the corresponding soil samples adjacent to the plants were composited by species only. The results of analyses of the composited samples (table 2) indicate that increases in size and presumably in age in both species are associated with increases in salt content and exchangeable-sodium percentage of the soil under the plants as well as with increases in pH as measured both in soil paste and in the 1:10 suspension. Also, the values obtained for these measurements were lower in the adjacent barren soil than under plants of any size. Soluble salts accumulated to a greater extent under shadscale than under greasewood, while the pH of bare soil was higher in the greasewood than in the shadscale area, presumably owing to the greater exchangeable-sodium percentage in the greasewood area.

TABLE 1

PH OF SURFACE SOIL UNDER AND BETWEEN INDICATOR PLANTS

Association	No. of paired samples	Av. field pH*		No. of paired samples	Av. laboratory pH†	
		Between plants	Under plants		Between plants	Under plants
Sagebrush	28	8.27	8.18	19	8.46	8.41
Shadscale	31	8.47	8.91	16	8.66	9.23
Greasewood	43	8.52	9.25	26	9.00	9.83

*pH of approximately 1:5 soil-water suspensions determined in the field with thymol blue and a spot-plate.

† pH of 1:5 or 1:10 soil-water suspensions determined by means of a glass electrode.

To determine more specifically the relation of plant size to variation in soil characteristics, pure greasewood associations were examined at locations in which greasewood shrubs of different sizes formed the corners of a square or the apices of an equilateral triangle whose centers were free of shrub growth. Chemical data from a typical location are given in table 3, and the values for exchangeable-sodium percentage are presented graphically in figure 1.

It is evident that pH, soluble-salt content, and especially exchangeable-sodium percentage increased with increase in

plant diameter, whereas the barren soil in the center of the triangle was relatively low in all these values at all depths. The pH_s and pH_{10} values were significantly greater under the plants of 12- and 24-inch diameters than in the barren soil at comparable depths. There was an accumulation of soluble salts in the surface layer under all plants regardless of size. The accumulation of salt, if any, was not significant below 2 inches under the 6-inch plant and below 6 inches under the 12-inch plant.

Soil samples were not taken to a depth sufficient to reveal whether the excessive accumulations of salt and exchangeable sodium under the greasewood plants originated in the subsoil. The root systems of these plants were not completely excavated, but it was determined that in all cases the taproot extended considerably deeper than the 12-inch sampling depth.

The data in table 4, which include the data in table 3, were assembled to show even more specifically the effect of size of greasewood plants on the chemical characteristics of soils of the Antelope Springs

TABLE 2

RELATION OF DIAMETER OF PLANT CROWN TO pH, SALT, AND EXCHANGEABLE SODIUM OF SOIL

	Bare soil	Shadscale diameter plants (in.)		Bare soil	Greasewood diameter plants (in.)		
		1–3	3–12		1–3	3–12	12–24
No. of samples composited	40	20	20	60	20	20	20
pH_s*	7.9	8.0	8.4	8.0	8.0	8.4	8.8
pH_{10}†	8.6	8.9	9.5	9.1	9.2	9.5	9.8
EC_e‡	0.7	1.1	6.1	0.8	1.1	2.4	3.8
ESP§				3.2	4.2	10.3	22.8

* pH of saturated paste.
† pH of 1:10 soil-water suspensions.
‡ Specific electrical conductance of saturation extract of soil in millimhos/cm at 25° C. See n. 6.
§ Exchangeable-sodium percentage. See n. 6.

TABLE 3

RELATION OF SOME SOIL CHARACTERISTICS TO DIAMETER OF PLANT CROWN AND SOIL DEPTH IN A PURE GREASEWOOD ASSOCIATION

Soil depth (in.)	pH_s				pH_{10}				EC_e (Mmhos/cm)				ESP			
	Crown diameter (in.)				Crown diameter (in.)				Crown diameter (in.)				Crown diameter (in.)			
	0*	6	12	24	0*	6	12	24	0*	6	12	24	0*	6	12	24
0–2	8.2	8.3	8.7	8.9	9.1	9.3	9.8	9.9	0.6	1.6	2.5	3.5	2.4	8.0	26.4	31.1
2–6	8.1	8.1	8.4	8.4	9.0	9.1	9.6	9.6	0.4	0.7	1.2	1.5	1.9	3.4	15.6	16.2
6–12	8.0	8.0	8.2	8.2	9.0	8.9	9.3	9.4	0.5	0.5	0.7	1.2	2.4	2.1	8.0	11.6

* 0— barren soil.

series. In some instances the sampling sites were several miles apart in noncontiguous areas of Antelope Springs soil; but the results obtained have been listed in order of increasing plant diameter without regard to location. Electrical conductivity, pH, and especially exchangeable-sodium percentage at each soil depth were positively correlated with the diameter of the crown and presumably the age of the greasewood plants under which the soil samples were taken.

It is logical to conclude that greasewood plants have not always occupied their present precise locations. This seems to be borne out by the fact that in no case were greasewood seedlings found under thriving greasewood plants or elsewhere in soils where the pH value was considerably higher than that of the barren soil. However, the bare areas and places where greasewood plants had formerly grown were not sampled in sufficient detail to establish whether the effect of greasewood on pH, exchangeable sodium, and soluble salts disappears rather quickly or persists for many years.

COMPARISON OF PH, ELECTRICAL CONDUCTIVITY, AND EXCHANGEABLE-SODIUM PERCENTAGE UNDER AND BETWEEN INDI-

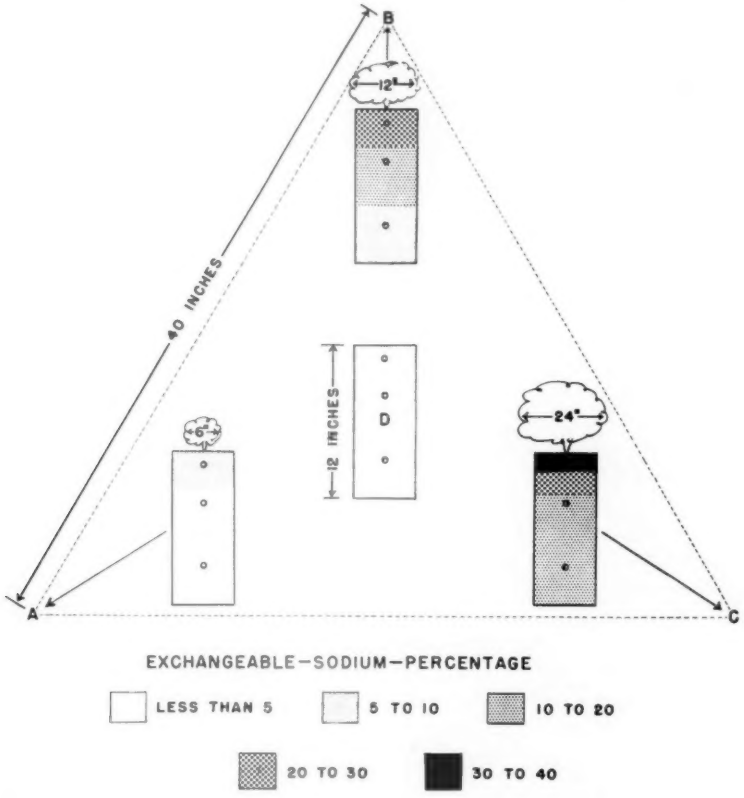

FIG. 1.—Relation of exchangeable-sodium percentage to diameter of plant crown and soil depth in a pure greasewood association.

CATOR PLANTS.—Data not reported here suggest that soil characteristics such as pH, soluble salt, exchangeable sodium, and permeability are not affected significantly by the growth of sagebrush, rabbitbrush, or pickleweed, but it has been shown that shadscale and greasewood do affect some of these soil characteristics. In the Beryl-Enterprise area these latter two shrubs were not found growing in close proximity except where the soil was quite heterogeneous, so that it was not possible to compare them on the same soil type. However, the effects of shadscale and greasewood were observed in separate areas of the Antelope Springs series. In addition, the relative effects of sagebrush and greasewood growing side by side in a fairly homogeneous area of this series were noted.

Table 5 gives data on the chemical characteristics of the Antelope Springs series as related to shadscale and greasewood. The barren soils of the two areas are similar in most respects, with that of the greasewood area having a slightly higher pH throughout and considerably more exchangeable sodium in the lower

TABLE 4

RELATION OF DIAMETER OF CROWN OF GREASEWOOD TO pH AND SALT AND SODIUM CONTENT OF SOIL

Soil depth (in.)	Diameter of crown (in.)							
	0*	6	9	12	24	36	44	54
	pH_s							
0–2	8.2	8.3	8.5	8.7	8.9	9.2	9.3	9.4
2–6	8.1	8.1	8.1	8.4	8.4	8.7	8.9	9.1
6–12	8.0	8.0	8.1	8.2	8.2	8.4	8.3	8.5
	pH_{10}							
0–2	9.1	9.3	9.7	9.8	9.9	10.1	10.1	10.2
2–6	9.0	9.1	9.2	9.6	9.6	9.9	10.0	10.0
6–12	9.0	8.9	9.3	9.3	9.4	9.7	9.7	9.7
	EC_e							
0–2	0.6	1.6	2.6	2.5	3.5	5.5	9.5	9.9
2–6	0.4	0.7	0.8	1.2	1.5	2.7	3.1	4.3
6–12	0.5	0.5	0.8	0.7	1.2	1.9	3.1	4.4
	ESP							
0–2	2.4	8.0	19.8	26.4	31.1	52.3	55.4	59.8
2–6	1.9	3.4	5.6	15.6	16.2	36.1	45.0	49.9
6–12	2.4	2.1	7.9	8.0	11.6	22.1	30.1	37.7

*0 = Barren soil. Values in this column are averages of eleven determinations taken in barren soil adjacent to plants of each size. All other values are averages of one to three determinations depending on plant size.

layers of the profile. As a result of the growth of shadscale and greasewood shrubs, pH of the paste and 1:10 suspension, electrical conductivity of the saturation extract, and exchangeable-sodium percentage were increased significantly at most depths. Increases in pH_s and pH_{10} in the top layers, in salt content in the lower layers, and in exchangeable-sodium percentage throughout the profile were greater under greasewood than under shadscale. However, except for exchangeable sodium, these increases do not appear to be important as regards plant growth. Exchangeable sodium accumulates to a limited extent under shadscale, and to a much greater extent and depth under greasewood, with consequent harmful effects upon permeability and soil tilth as indicated by permeability tests, moisture-retention data, settling volume, and wet sieving.

The data in table 6 show some of the differences in the characteristics of soil samples taken from the sides of a trench dug between adjacent sagebrush and greasewood shrubs of the same size and from the intermediate bare soil. A total of forty-five soil samples was taken from five depths at nine locations along the trench, three locations being under each shrub and three in the intermediate barren area. For brevity and ease of presentation, only the mean values for each determination under each shrub and from the bare area are listed. Figure 2 gives a schematic representation of the size and positions of the shrubs, the mid-points of

TABLE 5

RELATION OF SHADSCALE AND GREASEWOOD TO SOME CHEMICAL CHARACTERISTICS OF THE SOIL*

Soil depth (in.)	pH		pH_{10}		EC		ESP	
	Bare soil	Under plant	Bare soil	Under plant	Bare soil	Under plant	Bare soil	Under plant
	Shadscale							
0–1	7.8	8.2	9.0	9.5	0.9	3.9	4.1	18.0
1–5	7.9	8.0	8.8	9.4	1.1	2.2	3.6	13.0
5–12	7.6	7.2	8.7	8.3	0.5	1.4	2.8	3.8
12–22	7.7	7.6	8.7	8.6	0.4	1.1	1.7	1.9
22–33	7.6	7.5	8.6	8.6	0.9	1.2	1.5	1.6
33–44	7.7	7.6	8.8	8.8	0.9	1.0	3.8	2.8
	Greasewood							
0–1	8.3	8.6	8.8	9.7	0.7	3.8	2.3	27.8
1–6	8.0	8.7	8.9	9.8	0.6	2.7	2.2	38.8
6–15	8.1	8.5	8.9	9.6	0.4	2.5	3.6	26.8
15–23	8.2	8.2	9.1	9.0	0.6	3.0	8.4	12.2
23–38	8.3	8.3	9.4	9.4	0.8	2.5	22.9	25.1

* In the shadscale area each value is the mean of determinations on two separate samples taken underneath or adjacent to the same shadscale shrub. In the greasewood area each value for bare soil is from a single determination, and for soil under plants, each value is the mean of four determinations, each determination having been made on a soil sample from a separate sampling site under a single greasewood shrub approximately 30 inches in diameter.

the sample depths, and the approximate position of soil layers having equal exchangeable-sodium percentages.

It is evident that sagebrush had little, if any, effect on the soil properties measured. On the other hand, greasewood had a marked influence on these same properties, especially on the exchangeable-sodi-

TABLE 6

RELATION OF ADJACENT SAGEBRUSH AND GREASEWOOD PLANTS TO SOME CHEMICAL CHARACTERISTICS OF THE SOIL*

Soil depth (in.)	pH_s			pH_{10}			EC_e			ESP		
	Sage-brush	Inter-space	Grease-wood	Sage-brush	Inter-space	Grease-wood	Sage-brush	Inter-space	Grease-wood	Sage-brush	Inter-space	Grease-wood
0–2	7.9	8.2	8.8	8.8	9.1	10.1	2.0	1.0	6.0	2.6	3.2	41.3
2–6	7.9	8.0	8.6	9.0	9.1	9.9	0.8	0.7	2.8	2.9	2.5	33.9
6–16	7.9	8.0	8.3	9.0	9.0	9.7	0.9	1.0	2.5	3.7	2.0	26.7
16–25	8.0	8.1	8.2	9.1	9.2	9.5	0.6	0.9	2.5	8.9	8.5	26.5
25–32	8.2	8.4	8.2	9.3	9.4	9.5	0.6	1.0	3.3	14.4	19.7	30.2

* Each value is the mean of three determinations made on separate soil samples which were taken under sagebrush, under greasewood, and in the interspace. The plants were similar in height and crown diameter.

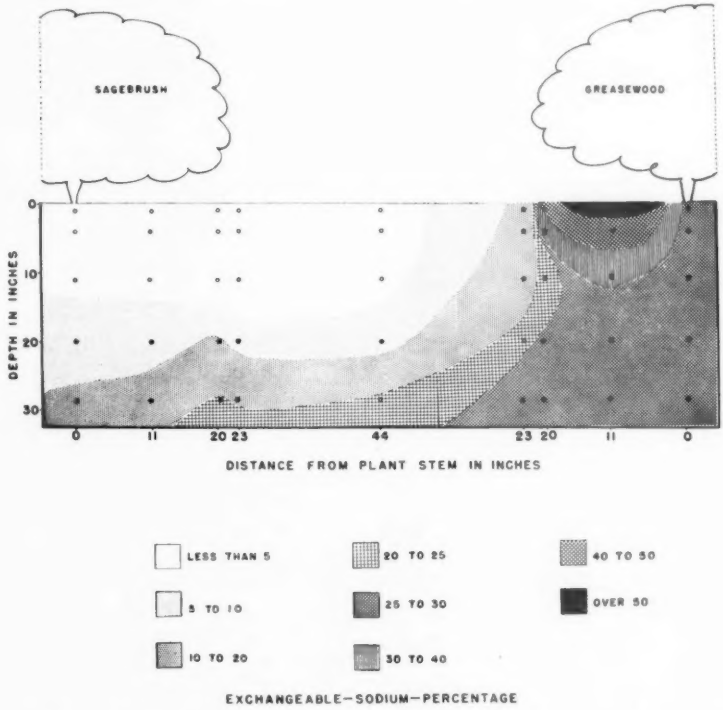

FIG. 2.—Exchangeable-sodium percentage of soil under sagebrush and greasewood plants and in the intervening barren area.

um percentage. The growth of greasewood resulted in an appreciable increase in pH_s (to a depth of 16 inches) and in pH_{10} (to a depth of 25 inches). There were very large increases in electrical conductivity and exchangeable-sodium percentage at all depths sampled. The highest values for all measurements were in the surface-soil layer underneath the greasewood plant, and generally the values decreased with depth. On these soils the higher exchangeable-sodium percentages were related to low permeability and poor tilth. In laboratory tests an excess of gypsum ($CaSO_4 \cdot 2H_2O$) was added to the surface of samples with low permeability, and then they were leached with a few inches of irrigation water. As a result of this treatment most of the exchangeable sodium was removed from the soil with consequent improvement of permeability and soil tilth.

In the absence of detailed knowledge as to the characteristics and distribution of the root systems of these plants at this location, the significance of the difference in the positions of the zones of equal exchangeable-sodium percentage under sagebrush and greasewood is conjectural. It is not likely that greasewood affects the exchangeable-sodium content of the soil much beyond the periphery of the crown. It is probable that this subsoil, and perhaps the entire soil profile, originally contained a relatively high concentration of exchangeable sodium. Subsequently, sodium accumulated under greasewood, but it appears to be decreasing in the barren soil and under such plants as sagebrush.

CATION AND ANION CONTENT OF INDICATOR PLANTS.—Roots, stems, branches, and leaves of greasewood, shadscale, sagebrush, rabbitbrush, and pickleweed were analyzed for their content of sodium, potassium, calcium, magnesium, chloride, and sulfate ions; and for malic, citric, oxalic, and total organic acids. The total ionic content of each plant species was positively correlated with the soluble-salt content of the soil, and the relative ionic proportions and concentrations were different for the various plant parts of each species. Moreover, these species exhibited large differences in the accumulation of both anions and cations when growing side by side in either saline or nonsaline soils. The most significant conclusion drawn from these data was that greasewood, which had the greatest effect on the soil properties measured, also had by far the greatest proportion of its anions in the form of organic acids.

A possible explanation for the accumulation of exchangeable sodium under greasewood plants, in particular, is provided by the high proportion of organic acid anions in its leaves. When these leaves drop to the ground, the organic matter slowly decomposes with the liberation of cations and anions and the formation of more or less soluble salts. These will be, initially at least, the sodium, potassium, calcium, and magnesium salts of chloride, sulfate, malic, citric, oxalic, and other organic acid anions. The organic acid anions decompose further to carbonate and bicarbonate anions. The carbonates and bicarbonates of calcium and magnesium are relatively insoluble and so they precipitate, leaving the soil solution composed almost entirely of sodium and potassium salts. These react with the exchange complex to displace calcium and magnesium and form soils that are higher in exchangeable sodium and potassium with consequent increase in pH and deterioration of soil structure.

Summary

1. Chemical effects of native plants on desert soils were studied.

2. Data are presented which indicate that the pH values of saturated soil pastes and 1:10 soil-water suspensions, particularly of the surface soil, generally are higher under shadscale and invariably are higher under greasewood than in the adjacent bare areas or under plants such as sagebrush.

3. The pH value of the bare soil is usually highest in greasewood and lowest in sagebrush areas, with shadscale areas either intermediate or similar to those of sagebrush.

4. The exchangeable-sodium percentage of the soil is somewhat higher under shadscale and very much higher under greasewood than in the adjacent bare areas or under other plants such as sagebrush.

5. The soluble-salt content of the soil was appreciably higher under shadscale and greasewood than in adjacent bare soil or under other shrubs.

6. The increases in pH, exchangeable-sodium percentage, and, to some extent, salt content of soil which result from the growth of greasewood are associated with the size and presumably the age of the plant. That is, the larger the plant, the higher the pH, soluble-salt content, and exchangeable-sodium percentage as compared with soil in adjacent bare areas or under plants such as sagebrush.

7. Chemical analyses of plant parts and organic litter in relation to the zonation of pH and exchangeable-sodium percentage are discussed; and a mechanism which would account for the increase in pH, soluble salts, and exchangeable sodium is proposed.

The authors wish to express appreciation to RAY ROBERTS and W. G. HARPER, Division of Soil Survey, BPISAE, the former for calling to our attention some of his observations regarding pH values under desert shrubs, the latter for his assistance in the location of sampling sites and with soil descriptions. Thanks are due to LORIN W. HEALTON of this laboratory for his assistance in both the field and the laboratory phases of this investigation; and to W. P. COTTAM, Professor of Botany, University of Utah, for his careful review of the preliminary draft of this paper.

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EFFECT OF NIGHT TEMPERATURE ON GROWTH OF THE FRUIT OF THE SOUR CHERRY

LOREN D. TUKEY

Introduction

Growth of the fruit of the sour cherry (*Prunus cerasus* L., var. Montmorency) occurs in three distinct stages (**17, 20**): (I) a period of rapid development of the fleshy pericarp beginning at the time of full bloom; (II) a period of retarded development of the fleshy pericarp; and (III) a second period of rapid development of the fleshy pericarp, commonly called the "final swell." This cyclic growth has been studied and found characteristic also of the apricot (**9**), peach (**4, 6, 7, 10, 14, 16**), plum (**11**), and sweet cherry (**13, 15**).

LILLELAND (**12**) has shown that temperature markedly affects the cyclic growth of the apricot fruit. He subjected growing apricots to different night temperatures by inclosing part of a limb of an apricot tree bearing developing fruits in a chamber in which the temperature was raised during the night. Results showed that for fruits outside the chambers the duration of stage I was 46 days; stage II, 44 days; and stage III, 28 days, totaling 118 days, while within the chambers at higher night temperatures stage I was shortened to 22 days; stage II lengthened to 49 days; and stage III shortened to 24 days, totaling only 97 days.

The experimental work reported in this paper was undertaken to determine the effect of night temperature on growth of the sour-cherry fruit.

Material and methods

The sour cherry was selected because of several desirable features: the growth pattern of the fruit has already been established (**17, 20**); a relatively short time is required for fruit to mature; and fruits are borne close together so that a large number can be confined in a small chamber. Furthermore, all flower buds, flowers, or fruits are in approximately the same stage of development at the same time, with little or no overlapping, thus eliminating complicating factors associated with a wide spread in time of flowering and other reproductive phases.

The studies were conducted during the springs of 1948 and of 1949 on 8-year-old trees in The Ohio State University orchard at Columbus, Ohio. Sufficient spraying was done to insure freedom from insect and disease troubles. The Montmorency cherry is both self-fertile and self-fruitful, but to insure pollination, flowers were hand pollinated. Each spring limbs at a convenient height from the ground were inclosed in two separate chambers in which the desired night temperatures were maintained.

Night-temperature treatments alone were used (*a*) because of the suggestions from the work of LILLELAND with the apricot (**12**) and of WENT with the tomato (**22**) and (*b*) to avoid introduction of factors associated with photosynthesis.

CONSTRUCTION AND OPERATION OF CHAMBERS.—The chambers (fig. 1) were approximately 3 feet on a side. The frame was made of 2 × 2-inch pine. The bottom of the chamber consisted of $\frac{3}{4}$-inch pine boards covered with a layer of $\frac{1}{4}$-inch asbestos board. The roof was made of a layer of roofing tarpaper, a layer of Celo-glass, 2 inches of Fiberglas insulation, and a second layer of Celo-glass. Three sides and the upper part of the fourth side of the chamber were removable doors. Door frames were constructed of 2 × 2-inch pine and were covered on both sides with a layer of Celo-glass between which was a 2-inch layer of Fiberglas insulation. The lower part of the fourth side was a $\frac{3}{4}$-inch pine board in which a slot was made at an appropriate location to receive and hold the branch inserted in the chamber. The chamber was mounted on wooden posts set 2 feet in the ground.

FIG. 1.—Temperature chamber with inclosed sour-cherry limb. Doors lying under chamber were installed in evening and removed in morning.

A 60-foot length of lead heating coil of the type commonly used in plant propagation benches was spread over the bottom of the chamber as a source of heat. Temperatures were controlled by a liquid-expansion thermostat containing a mercury switch and connected through a relay. The expansion tube was placed in approximately the middle of the chamber, the same distance above the heating coils in both chambers. An electric fan, with blades bent so as to produce only slight air circulation, was placed in the left rear corner to insure uniform air temperature throughout the chamber. The fan was tilted so that the air stream passed around the thermostat expansion tube. A thermograph was placed above the heating coil on the right rear of the chamber to record temperatures continuously. Outside air temperatures were recorded on a thermograph placed near by in a standard shelter. Thermographs were adjusted before the experiment and checked at least twice a day against a mercury thermometer. All temperatures were recorded on the Fahrenheit scale. There were sufficient small cracks and openings in the chamber to permit free movement of carbon dioxide and other gases from and into the chambers during the night.

The doors were placed on the chambers and the temperature raised at approximately 7:00 P.M. each evening. Doors were removed and temperature lowered between 7:00 and 8:00 A.M. each morning. The electric fans and thermostat controls were operated continuously during the night. Temperature settings of the thermostats were determined each night from the average outside night temperature of the previous night and from personal observations of weather

conditions. Nightly adjustments of the thermostats were required.

The temperature treatments were designated: CK_1, check temperature treatment in 1948; CK_2, check temperature treatment in 1949; M, medium temperature treatment in 1948; H_1, high temperature treatment in 1948; H_2, high temperature treatment in 1949; and VH, very high temperature treatment in 1949. In 1948 the M chamber was set approximately 10° F. above the average outside night temperature, and the H_1 chamber approximately 20° F. above this average. In 1949 the H_2 chamber was set approximately 20° F. above the average outside night temperature, and the VH chamber approximately 25° F. above this average. Treatments were started immediately after full bloom and continued beyond the end of stage III.

METHOD OF EXPRESSING TEMPERATURE.—Hourly temperatures were obtained from the recorded temperature charts from full bloom to the completion of stage III. Only temperatures during the hours of nightly temperature treatments were used, that is, from 7:00 P.M. to 7:00 or 8:00 A.M. The average for a night was determined by adding hourly night temperatures and dividing by the number of hours. Average night temperature for a given stage was determined by adding average temperatures per night for the period indicated and dividing by the number of nights involved. Results are expressed and compared on the basis of average night temperatures for a definite period of either one, two, or three stages. That is, the average night temperature for a stage covered just the duration of that stage; that at the completion of a stage covered the period from full bloom to the completion of that stage.

SAMPLING AND GROWTH MEASUREMENTS.—As soon as flowers showed evidence of having been fertilized, ten young fruits in each treatment were selected at random, numbered 1 to 10, and measured every 2–3 days throughout the growing season until after maximum size was reached. Cheek diameter, suture diameter, and fruit length were measured. Additional measurements were made at critical points in fruit development, such as the transition from one stage to another.

For critical studies of embryo, endosperm, and nucellus and integuments, representative samples of fruit were collected every 3 or 4 days and fixed in a solution containing 90 ml. of 50% ethyl alcohol, 5 ml. of propionic acid, and 5 ml. of formalin.

As an estimate of the total soluble solids in the fruits, refractometer readings were made at the end of the 1949 season for all treatments by means of an Abbé refractometer. All readings were calculated as of 20° C. and expressed as per cent sucrose.

Differences in leaf color were determined on samples containing fifty leaves by using a Photovolt photoelectric meter (**8**). This apparatus measures the amount of reflection of light from a test surface as compared with that of a standard composed of a freshly prepared magnesium oxide surface with a reflectance taken as 100%. By use of a Tri-Green filter the relative amount of light and darkness of a green surface could be readily measured, thus giving differences in shades of green.

Results

TYPICAL DEVELOPMENT

Typical growth of the Montmorency sour cherry in Ohio during the seasons of 1948 and 1949 was found (fig. 2 and table 1) to be similar to that in New York as reported by TUKEY (**17, 20**). Stage I ex-

tends from the time of full bloom to the hardening of the stony pericarp. During the prebloom stage and the first half of stage I the increase in size of the fleshy pericarp is largely by cell division. During the second half of stage I the cells approximately double in diameter. The stony pericarp increases in size by cell division during the prebloom stage and the first few days of stage I and by cell enlargement during the latter part of this stage.

Stage II extends from the conclusion of stage I to the beginning of the second period of rapid development of the pericarp. During stage II there is a slight enlargement of the fleshy pericarp; the cell walls of the stony pericarp become further thickened and greatly hardened. A feature of this stage is the rapid growth of the embryo from microscopic size to maximum size of 6.0–6.5 mm. in length.

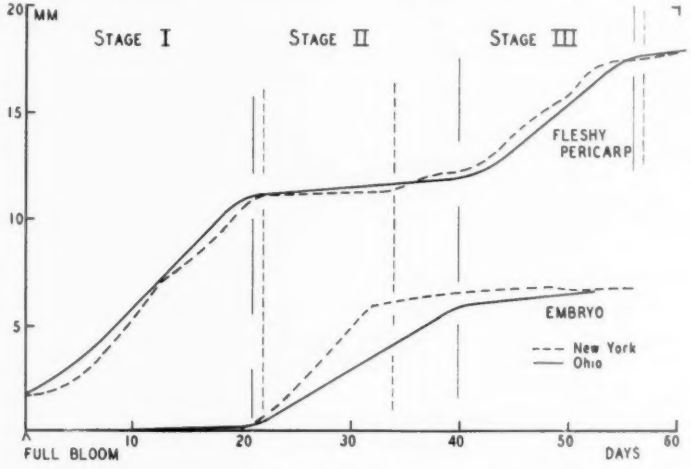

FIG. 2.—Comparison of growth curves for Montmorency sour cherry in New York (17) and in Ohio, average for 1948 and 1949.

TABLE 1

NUMBER OF DAYS TO COMPLETE STAGES IN FRUIT DEVELOPMENT OF MONTMORENCY SOUR CHERRY IN OHIO AND IN NEW YORK (17)

LOCATION	DURATION IN DAYS			
	Stage I	Stage II	Stage III	Total
New York...	22	12	23	57
Ohio, 1948...	21	19	16	56
Ohio, 1949...	18	18	18	54

Stage III, often called the "final swell," extends from the conclusion of stage II to fruit maturity. During this stage the fleshy pericarp increases greatly through cell enlargement. The largest cells at maturity have increased 250 times from their diameter at the time of full bloom. The stony pericarp becomes slightly harder and more brittle. The embryo increases only slightly in size. The morphological changes that occur are primarily in the fleshy pericarp.

EFFECT OF NIGHT TEMPERATURE ON DURATION OF EACH STAGE AND TO MATURITY

An increase in the average night temperature resulted in the shortening of

stage I (fig. 3; table 2). The duration of stage II also was decreased as the average night temperature was raised. In contrast, an increase in average night temperature during stage III resulted in an increase in its duration rather than a decrease as in stages I and II.

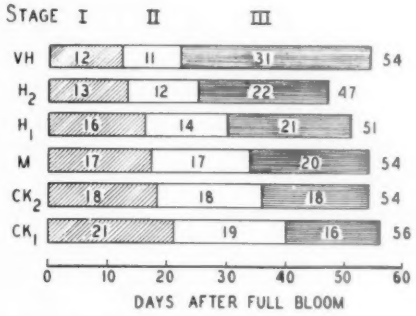

FIG. 3.—Duration of each of three stages in development of fruit of Montmorency sour cherry at six different night temperatures shown in table 2.

and VH treatments even though the seasonal average night temperature for the M treatment was 5°.7 F. higher than for the CK_2 treatment, and though the VH treatment was in turn 22°.6 F. higher than the M treatment. However, the number of days for their stages varied significantly owing to the differing effect of temperature on the duration of each of the three stages. For example, at the highest temperature (VH treatment) the decrease in number of days for completion of stages I and II was more than offset by the lengthening of stage III, thus resulting in a relatively long period from full bloom to maturity.

MORPHOLOGICAL CHANGES

FLESHY PERICARP.—In all treatments the fleshy pericarp increased rapidly in size immediately following full bloom

TABLE 2

NUMBER OF DAYS TO COMPLETE STAGES IN FRUIT DEVELOPMENT OF MONTMORENCY SOUR CHERRY GROWN AT SIX DIFFERENT NIGHT TEMPERATURES

TEMPERATURE	STAGE I		STAGE II		STAGE III		TOTAL	
	Days	Av. night temp. (°F.)	Days	Av. night temp. (°F.)	Days	Av. night temp. (°F.)	Days	Av. night temp. (°F.)
CK_1 (check, 1948)	21	54.5	19	53.7	16	62.0	56	56.4
CK_2 (check, 1949)	18	54.9	18	56.5	18	66.9	54	59.4
M (medium, 1948)	17	58.5	17	66.6	20	69.4	54	65.1
H_1 (high, 1948)	16	65.6	14	74.9	21	76.5	51	72.6
H_2 (high, 1949)	13	74.6	12	79.9	22	84.9	47	80.8
VH (very high, 1949)	12	79.5	11	87.8	31	91.2	54	87.7

In summation of all three stages, for each succeeding increase in temperature through 80°.8 F. there was a decrease in the number of days to maturity (table 2). On the other hand, at a seasonal average night temperature of 87°.7 F., the number of days to maturity was greater than at 80°.8 F.

The number of days from full bloom to maturity was identical for the CK_2, M, and continued to the completion of stage I. However, the rate of growth of the fleshy pericarp indicated by the slope of the growth curve (fig. 4) was greatest at the highest night temperature (VH treatment). At the completion of stage I the average diameter of the fleshy pericarp was somewhat similar in all treatments, especially so when the data are considered for each season separately. How-

ever, night temperatures in the VH treatment apparently inhibited fruit enlargement significantly, but further treatments seem necessary to substantiate this. The transition from stage I to II was abrupt in the CK_1, CK_2, M, and H_1 treatments but was less so with further increases in night temperature (H_2 and VH treatments). In fact, for the VH treatment, so gradual was the change that this transition was difficult to determine from fleshy pericarp measurements.

During stage II the average diameter increase of the fleshy pericarp was 0.6 mm. to 1.3 mm., the greatest increase being in the VH treatment (table 3). However, there was more uniformity in fruit size among the treatments at the

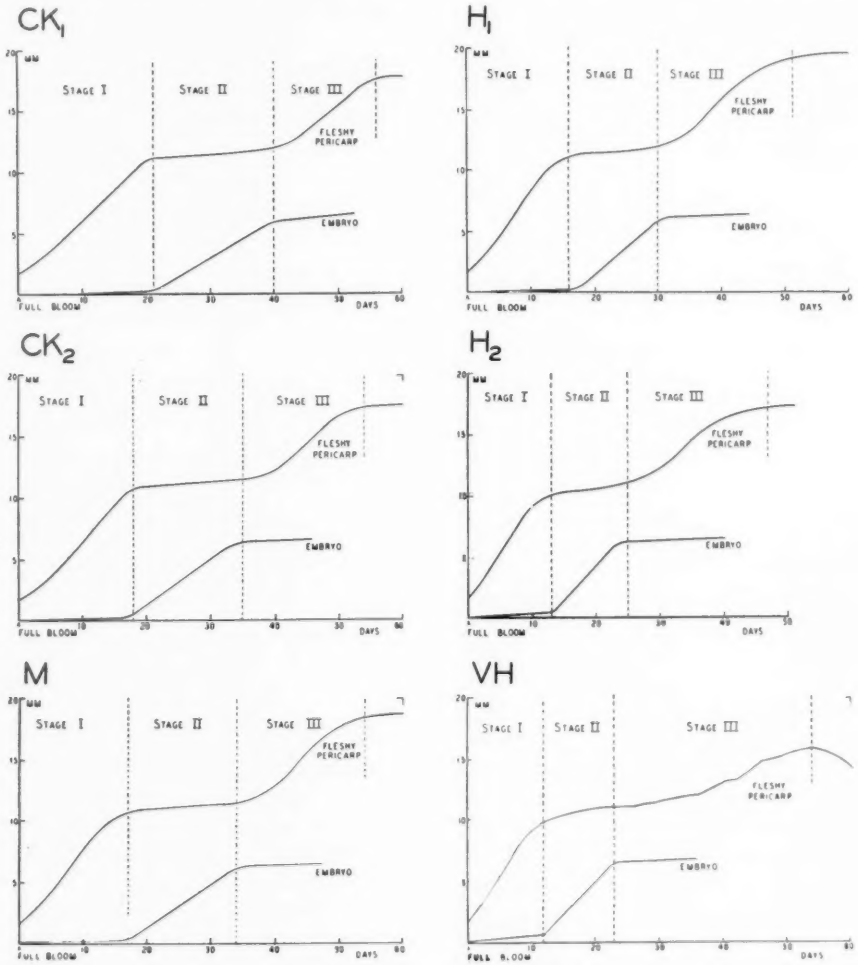

FIG. 4.—Growth curves for fruit of Montmorency sour cherry at six different temperature conditions. CK_1 check, 1948; CK_2, check, 1949; M, medium, 1948; H_1, high, 1948; H_2, high, 1949; VH, very high, 1949.

completion of stage II than at that of stage I. This seemed to indicate that night temperature had relatively little effect on fruit size up to this point. The fruit attained an average diameter ranging from 11 to 12 mm. (table 3) at the completion of stage II.

The transition from stage II to III was gradual, more so than that from stage I to II. In fact, considerable difficulty was encountered in establishing the point for the completion of stage II solely by use of the growth curve of the fleshy pericarp. In the VH treatment the slope of the curve, established during stage II, continued almost halfway through stage III. Hence the completion of stage II was determined entirely by embryo growth.

During stage III the fleshy pericarp increased in size rapidly. The slope of the curve was less at the higher temperatures with a longer duration of stage III. This is evident when comparing the CK_1 and VH treatments (fig. 4). The growth curve for the fleshy pericarp at the end of stage III leveled off in all conditions except the VH treatment. Here the growth curve reached a maximum point and then decreased at a rate similar to that just before maximum size was reached. The M and H_1 treatments not only had the largest fruits but also showed the greatest size increases in average diameter, 7.0 mm. and 7.3 mm., respectively (table 3). The smallest fruits were found in the VH treatment where the high night temperature was evidently a factor in limiting the average diameter increase during stage III to only 4.8 mm.

STONY PERICARP.—The growth of the stony pericarp paralleled that of the fleshy pericarp during stage I, regardless of the temperature treatment, and this region reached maximum size at the completion of this stage. Likewise, hardening had commenced by the beginning of stage II and continued progressively to fruit maturity.

TABLE 3

AVERAGE DIAMETER OF FLESHY PERICARP AND LENGTH OF EMBRYO OF MONTMORENCY SOUR CHERRY AT THE COMPLETION OF EACH STAGE OF FRUIT DEVELOPMENT

TEMPERATURE	AVERAGE DIAMETER OF FLESHY PERICARP (MM.)			EMBRYO LENGTH (MM.)
	At completion of stage I	At completion of stage II	At completion of stage III	At completion of stage II
CK_1 (check, 1948)	11.0	11.9	17.7	6.0
CK_2 (check, 1949)	10.9	11.5	17.4	6.5
M (medium, 1948)	10.7	11.4	18.4	6.3
H_1 (high, 1948)	11.3	12.0	19.3	6.2
H_2 (high, 1949)	10.0	11.0	17.0	6.2
VH (very high, 1949)	9.7	11.0	15.8	6.5

NUCELLUS AND INTEGUMENTS.—The nucellus and integuments increased rapidly in size during stage I, reaching maximum size by the end of this stage. In all treatments the growth of the nucellus and integuments paralleled that of the fleshy pericarp.

EMBRYO.—The embryo in all treatments remained microscopic during stage I and commenced to increase rapidly in size at the completion of this stage. Its approximate maximum length was attained at the end of stage II (fig. 4); the average was 6.3 mm. (table 3). The rate

of embryo growth, indicated in figure 4 by the slope of its growth curves, was positively correlated with night temperature. A comparison between the CK_1 and VH treatments is illustrative. The duration of the period of rapid lengthening paralleled the duration of stage II, since embryo development was used as a criterion in determining the duration of this stage. No appreciable difference was found in embryo length among the treatments (table 3). Consequently, night temperatures seemed to have little effect on embryo development other than on its rate of growth.

Leaf color

Higher night temperatures resulted in leaves with less green color. For example, the percentage of reflectance taken in 1949, 58–59 days after full bloom, was: CK_2, 7.3; H_2, 8.6; and VH, 11.0. Leaves receiving the VH treatment were visibly yellowish green.

Chemical analysis

Refractive index readings were made in 1949, 59–60 days after full bloom, of the liquid fraction of the fleshy pericarp. The percentage of total soluble solids expressed as sucrose were as follows: CK_2, 13.41; H_2, 11.01; and VH, 9.71, thus indicating a lower amount of total soluble solids with higher temperatures. Cherries receiving the VH treatment were, at the time of analysis, small, poor in color, and somewhat shriveled. Some of them were light red to pink, while others were yellow with a pink blush. Cherries in the other treatments were a natural red color.

Discussion

Went (**22**) has reported temperature requirements for growth of the tomato and pepper. Optimum growth was obtained at a day temperature of 26° C. for the season. The optimum night temperature dropped from 30° C. for young plants to 18° C. for full-grown plants. This gradual decrease in optimum night temperature was also found for blossoming, fruit-set, and fruit development.

The results reported here indicate a similar trend for fruit growth of the Montmorency cherry. The night temperature that produced the shortest duration of each stage in fruit development retrogressed from stages I and II to stage III. The average night temperature for each of these stages was: stages I and II, respectively, 79°.5 F. and 87°.8 F.; and stage III, 62°.0 F. These were the highest average night temperatures experienced in stages I and II and the lowest in stage III. The optimum day temperature for growth was not determined. However, the differential between night and day temperatures was noted. The most effective night temperatures during stages I and II were higher than the prevailing day temperatures, while during stage III they were lower than day temperatures. This trend was similar to the results reported on the tomato and pepper (**22**).

The experimental results also substantiated the observations made by others of temperature influences on several fruits (**1, 2, 3, 5, 12, 19, 21**). Warm temperatures immediately following full bloom or, in this case, during stage I bring about a decrease in the number of days to fruit maturity. A similar effect was also noted here for stage II. However, warm temperatures late in the season (stage III) noticeably lengthen the number of days to maturity.

Irrespective of the temperature treatment, the cyclic pattern of cherry fruit growth prevailed. Further, all parts of the fruit grew in apparently the same relation to each other regardless of the

temperature differential. The size (average diameter) of the cherries at maturity did not differ significantly except under the highest night temperature conditions (VH), where the fruits were smaller. The maximum length of the embryo was not affected in any way.

The poor color and low total soluble solids of fruit growing under very high night temperatures are factors in determining the distribution (and adaption) of successful cherry production. Cool night temperature during stage III was conducive to good color of fruit and high soluble solids. The undesirable effect of a high night temperature during stage III was also shown by the yellowish-green color of the leaves on the limbs so treated. Photosynthetic activity was undoubtedly reduced; this may have been one of the factors contributing to the poor color of fruit developing under these conditions. The undesirability of growing sour cherries in warm climates is evident. The areas of greatest production for the sour cherry in North America are located where cool night temperatures prevail during stage III of fruit development, though this is not the only factor determining the distribution.

The results indicated that warm night temperatures during stages I and II are essential for production of a quality commercial crop of cherries in the shortest possible time. Conversely, night temperatures during stage III must be low. Theoretically, it should be possible to mature cherries in 39 days by providing high temperatures for stages I and II and low temperatures for stage III comparable to those allowing most rapid development of these stages in these experiments. A still further reduction in the number of days to maturity might be possible by providing still higher temperatures during stages I and II and lower temperatures during stage III.

In these experiments differences in the number of days to fruit maturity (fig. 3) varied from 2 to 9 days depending upon the night temperature treatment. However, the degree of reduction in the number of days to maturity brought about by high average night temperatures during stages I and II does not appear in the actual number of days to maturity since the effect of similar high temperatures during stage III offset this earlier reduction. Consequently, there was an equalization in the final effect of night temperatures on the duration of fruit development.

Therefore, within a given locality or in many cases among localities where the night temperatures from one season to the next do not fluctuate to the extent actually produced in these experiments, the number of days to maturity will be about the same from one season to the next and somewhat similar under extremes. This conclusion is partly based upon the fact that average night temperatures continuously become warmer as the growing season progresses. If the duration of stage III was inversely related to the average night temperature, there would be a considerable variation in the number of days to maturity for a given locality.

These results would also tend to explain in part why differences in harvesting dates of a crop for a given region are frequently less than those at the time of planting or of bloom. Night temperatures following planting or bloom would be an influencing factor in this case.

Summary

1. The effect of night temperature was determined for the growth of sour-cherry fruits (*Prunus cerasus* L., var. Montmorency) during two growing sea-

sons, 1948 and 1949. Fruiting limbs of cherry trees were confined at night in heated chambers: Six temperature conditions were provided, designated CK_1, CK_2, M, H_1, H_2, and VH, with average night temperatures, respectively, of 56°.4, 59°.4, 65°.1, 72°.6, 80°.8, and 87°.7 F.

2. Cyclic growth curves for the sour cherry in Ohio were found to agree very closely with those determined in New York.

3. The duration of stages I and II was inversely related to night temperature.

4. The duration of stage III was positively correlated with night temperature.

5. Low total soluble solids, poor fruit color, and poor foliage color were produced with high night temperatures during stage III.

6. The total number of days to maturity was approximately the same under different night temperature conditions.

7. The growth correlations of developing parts of the sour-cherry fruit are not altered by night temperature changes.

8. Major commercial areas of sour-cherry production are located in regions that have cool night temperatures during stage III.

The author wishes to express his appreciation to Dr. FREEMAN S. HOWLETT, of The Ohio State University, for helpful suggestions and for providing the plant material and equipment necessary for the experiment, and to Dr. H. B. TUKEY, of Michigan State College, for suggesting the problem and for help and guidance.

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EFFECTS OF POTASSIUM DEFICIENCY ON GROWTH AND METABOLISM OF SUNFLOWER PLANTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 637

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Introduction

Higher plants require relatively large amounts of potassium; yet it is not used as a building stone for any indispensable organic compound. Although various functions in the plant have been ascribed to the element, there is considerable disagreement as to what roles it plays, and the mechanism of its action in any specific physiological process has not been satisfactorily explained.

In the case of the tomato two distinct types of plants may result from potassium deficiency (**21, 29, 30**). Early in the deficiency the plant resembles a nitrogen-deficient plant; it is chlorotic, hard and woody, and high in carbohydrates. Later, the leaves turn green, the carbohydrates decrease greatly, and the new growth is relatively soft.

There are few, if any, references in the literature to potassium-deficiency work on the sunflower plant. In classwork under the supervision of the writer, however, the two types of growth have not been found. When grown from seed without potassium, plants soon became chlorotic, and growth stopped at a height of a few inches. The second stage characterized by resumption of growth and greenness of leaves did not occur. Hence, it was thought that an extensive study of potassium deficiency in the sunflower might show interesting contrast with the tomato. Also, since the plant grows tall, it would be possible to analyze the stems at several levels. Significant data on the breakdown of organic compounds, reutilization of resulting materials, and functions of the element in the sunflower plant might be obtained.

Material and methods

Sunflower plants (*Helianthus annuus* L., var. Mammoth Russian) were grown in pure quartz sand by essentially the same methods as previously described (**3**). The complete nutrient solution was solution number 1 of Hoagland and Arnon (**14**). In the deficient solution sodium was substituted for potassium. The classwork mentioned above and preliminary work of the present experiment

[1] This research was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

indicated that, in the sunflower, sodium does not substitute for potassium and that there is little growth if the substitution in the nutrient solution is complete. Figure 1 shows plants grown with and without potassium at an age of 29 days. The former plants averaged 14.1 inches tall, the latter 4.5.

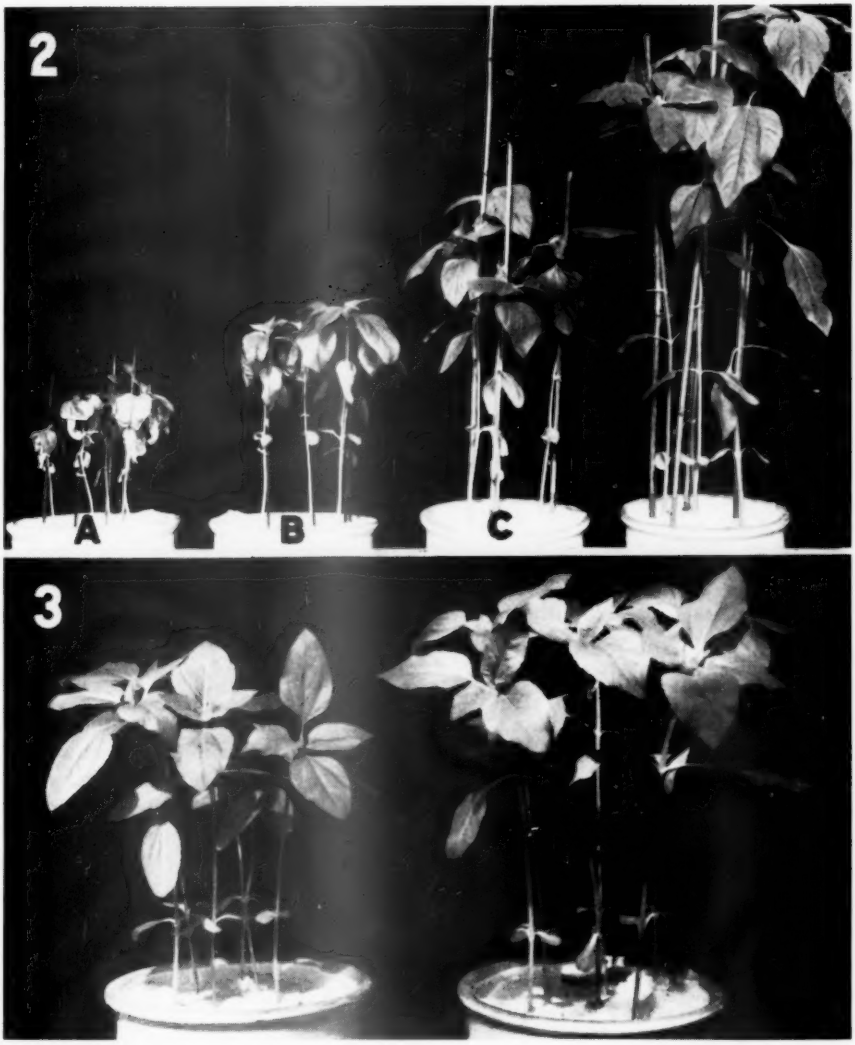
The above-mentioned experiment indicated that sufficient tissue for analysis would not be obtained in a potassium-deficient series if the plants received no potassium from the beginning of an experiment. Considerable preliminary work was done, therefore, to determine the length of time plants would have to be grown with complete nutrient before transfer to the minus-potassium schedule (fig. 2). It was found that size was proportional to the length of time on complete nutrient. The weight of the tops of four plants of the *A*, *B*, *C*, and *D* series was, respectively, 8.5, 16.4, 18.0, and 23.7 gm.

FIG. 1.—Sunflower plants, 29 days after planting of seed: *left*, minus-potassium; *right*, plus-potassium.

As a result of findings of this preliminary experiment all plants were grown for 11 days with the plus-potassium solution diluted to one-half strength for the first week. Then two-thirds of the pots were flushed thoroughly with distilled water on 3 successive days, the minus-potassium solution being added immediately afterward.

Seed was planted on May 1, 1951, and plants were harvested on May 24, May 31, and June 11. The number of plus- and minus-potassium plants of the first harvest were 71 and 142, respectively; of the second, 40 and 117; and of the third, 40 and 113. The first harvest was made before marked deficiency symptoms other than a size difference had appeared. Potassium-starved plants were somewhat greener than those on complete nutrient, but there was no chlorosis except in a few of the cotyledons. PHILLIPS *et al.* (**23**) stated that the primary effects of potassium deficiency might be indicated by comparing the chemical composition of plants just beginning to show the deficiency with that of controls receiving complete nutrient. Misleading results are obtained when plants suffering from extreme deficiency are analyzed. Yet the chemical composition of plants at an advanced stage of deficiency is of interest and may be similar qualitatively to that of plants at an early stage. The difference may be only quantitative. In recent work by the writer (**4**) tomato plants suffering from extreme sulfur deficiency were high in ammonia, amides, nitrates, sucrose, and starch but low in reducing sugars in comparison with those on complete nutrient. These same differences were present at an early stage of the deficiency but in a much reduced degree.

The methods used in sampling, extraction, and chemical analysis were essentially the same as previously described (**3**). Sodium hydroxide was used for obtaining alkalinity in the determination of ammonia, amides, and nitrates. Potassium was determined microchemically by the methods of ECKERSON (**5**), platinum chloride and sodium cobaltinitrite being



FIGS. 2, 3.—Sunflower plants. Fig. 2, 34 days after planting of seed: *A*, one-half strength complete nutrient for 1 week; *B*, same as *A*, then 3 days on full-strength complete nutrient; *C*, same as *A*, then 1 week on full-strength complete nutrient; *D* (*right*), same as *A*, then on full-strength complete nutrient to end of experiment. After treatment indicated, *A*, *B*, and *C* put on minus-potassium. Fig. 3, first harvest, 23 days after planting of seed: *left*, minus-potassium; *right*, plus-potassium.

the reagents used. A 50-ml. aliquot of the alcoholic extract was freed of alcohol, concentrated by evaporating to 10 ml., and filtered. One drop of this solution and one of the reagent were then transferred to a slide, mixed, and a cover glass was added. After standing a few minutes, the preparation was examined microscopically.

Results

APPEARANCE AND SIZE OF PLANTS.—Five days after beginning to withhold potassium the leaves of many minus-potassium plants appeared greener than those of the plus-potassium plants. Little difference was yet apparent in size of plants of the two series. Difference in greenness gradually became more pronounced and rather general, but at no time did potassium deficiency cause as marked greenness of sunflower leaves as in the case of phosphorus deficiency (**3**). Later, lower leaves of the minus-potassium plants became chlorotic, and chlorosis was followed by necrosis of the tissue. The usual pattern of chlorosis was a decrease in chlorophyll at tips and apical edges of the lower leaves and then extension of the chlorosis inward and toward the base. Veins remained green longer than the interveinal areas. Accompanying chlorosis the tip and edge of the leaf turned upward, and often there was twisting of the tip.

Minus-potassium plants were stunted in comparison with those receiving potassium. They had smaller leaves and shorter, thinner stems. The size differences were very great at later stages of deficiency (table 1, figs. 3–5). Figures 4 and 5 are of the same plants with a 10-day interval. Average heights of phosphorus-starved and of complete-nutrient plants shown in figure 4 were 12.4 and 26.5 inches, respectively; of figure 5, 13.4 and 41 inches. In the 10 days the latter plants grew greatly in height, the former very little, although they increased considerably in weight (table 1). No anatomical work was done, but to the touch and in cutting with a knife no difference in hardness of the stems of the two groups was noticed.

FRESH WEIGHT, PERCENTAGE WATER, AND PERCENTAGE DRY WEIGHT.—Given parts of minus-potassium plants weighed less fresh than those of plus-potassium plants, the differences being very great at the second and third harvests (table 1). Potassium deficiency affected tops more than roots, and growth of stems was checked more than that of leaves. The top-root, fresh-weight ratios for first, second, and third harvests were 4.146, 5.033, and 4.565, respectively, for potassium-starved plants; and 5.616, 7.631, and 8.295 for plants grown with complete nutrient. Corresponding leaf-stem, fresh-weight ratios were 1.063, 1.220, and 0.744 for the minus-potassium plants; and 0.726, 0.509, and 0.364 for those receiving potassium. Some leaves of the no-potassium plants had died by the third harvest. This increased the difference between top-root ratios and decreased that between leaf-stem ratios. Therefore, representative effects of the deficiency are shown by ratios of the first and second harvests.

Potassium-deficient leaves had lower water content (higher dry weight) than plus-potassium ones, and in each group the leaves contained less water than the corresponding stems. Minus-potassium stems were somewhat lower in water than the controls at the first and second harvests except at the lower level of the first harvest, but at the third harvest this was true only at the upper level. Calculated for whole stems the percentages of water of the no-potassium and complete-nutrient stems were 94.2 and 95.0, respectively, for the first harvest; 93.7 and

95.3 for the second; and 93.4 and 93.6 for the third.

TOTAL SOLIDS, TOTAL NITROGEN, SOLUBLE NITROGEN, AND INSOLUBLE NITROGEN.—Chemical constituents were figured on both dry- and wet-weight bases. To save space, however, only the former data are given, since the results are generally parallel.

Soluble solids were consistently lower in leaves than in stems, at least upper stems, of each group of plants (table 2). In general, they were higher in plus-potassium leaves and stems than in minus-potassium ones, although in certain cases the differences were not large enough to be significant. Stem percentages declined downward in each set of plants except in potassium-starved stems of the first harvest, and the values for these also declined downward calculated on a wet-weight basis.

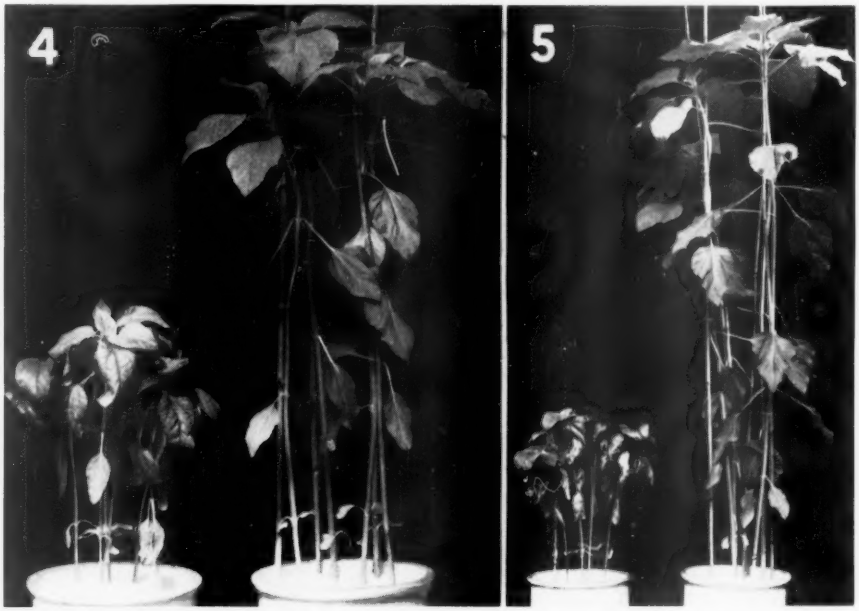
Total nitrogen was considerably higher and soluble nitrogen lower in the leaves than in the stems of each group of plants. In other words, soluble nitrogen made up a much lower percentage of total nitrogen in leaves of both potassium-starved and complete-nutrient plants than in their stems. In general, both total nitrogen and soluble nitrogen were higher in plus-potassium leaves and stems than in the minus-potassium ones, although differences were quite small in certain cases; at the lower stem level of the first harvest, and at the middle and

TABLE 1

FRESH WEIGHT (GM/100 PLANTS), PERCENTAGE WATER, AND PERCENTAGE DRY WEIGHT

PLANT PART	FRESH WEIGHT		PERCENTAGE WATER		PERCENTAGE DRY WEIGHT	
	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K
	First harvest (24-day-old plants)					
Leaves	327	466	88.9	90.6	11.1	9.4
Upper stems	101	203	93.8	95.1	6.2	4.9
Middle stems	93	206	94.0	95.4	6.0	4.6
Lower stems	115	233	94.8	94.6	5.2	5.4
Roots	153	197	.....	.....	.....	.....
	Second harvest (31-day-old plants)					
Leaves	645	1049	87.2	89.4	12.8	10.6
Upper stems	166	596	93.0	95.1	7.0	4.9
Middle stems	158	548	94.2	95.3	5.8	4.7
Lower stems	205	916	93.8	95.4	6.2	4.6
Roots	233	407	.....	.....	.....	.....
	Third harvest (42-day-old plants)					
Leaves	529	2394	85.2	87.9	14.8	12.1
Upper stems	205	1504	92.1	93.6	7.9	6.4
Middle stems	218	2190	94.2	94.0	5.8	6.0
Lower stems	289	2871	93.8	93.4	6.2	6.6
Roots	272	1080	.....	.....	.....	.....

lower levels of the third harvest, percentages favored the latter stems. Differences in insoluble nitrogen were small and not entirely consistent. In most cases, however, percentages were larger in potassium-deficient plants. In general, percentages of all nitrogen fractions declined downward in plus-potassium lower stems of the second harvest. In certain cases ammonia percentages of no-potassium leaves and stems were higher than the corresponding amide percentages. This may indicate that some glutamine was hydrolyzed during the extraction process. By combining ammonia and amide totals and calculating for whole



FIGS. 4, 5.—Sunflower plants. Fig. 4, at second harvest, 30 days after planting of seed: *left*, minus-potassium; *right*, plus-potassium. Fig. 5, at third harvest, 41 days after planting of seed: *left*, minus-potassium; *right*, plus-potassium.

stems. This was also true of minus-potassium stems in insoluble nitrogen but not consistently so in the other nitrogen fractions. At the third harvest the percentages of both total and soluble nitrogen were higher upward in the latter stems.

Ammonia and amides were consistently higher in minus-potassium leaves and stems than in plus-potassium ones (table 3). No ammonia was found in the latter leaves, in stems of the first harvest, or in stems, percentages for minus- and plus-potassium stems were 0.049 and 0.017, respectively, for the first harvest; 0.074 and 0.016 for the second; and 0.082 and 0.037 for the third. Nitrates were higher in most cases in the complete-nutrient leaves and stems than in the potassium-starved ones. Exceptions were lower stems of the first harvest, middle and lower stems of the third harvest, and leaves of the third harvest. When calculated for whole stems, nitrate percentages

for minus- and plus-potassium stems were 2.249 and 2.696, respectively, for the first harvest; 2.073 and 2.947 for the second; and 1.756 and 1.804 for the third. The nitrate values in the former stems were higher upward, but in the latter there was no definite gradient, middle potassium leaves and stems throughout the experiment than in plus-potassium ones. It made up a higher percentage of total sugars in the former stems than in the latter at the first harvest, these differences becoming greater at later harvests. In the leaves, however, sucrose

TABLE 2

SOLUBLE SOLIDS, TOTAL N, SOLUBLE N, AND INSOLUBLE N
(PERCENTAGES ON DRY-WEIGHT BASIS)

PLANT PART	70% ALCOHOL-SOLUBLE SOLIDS		TOTAL N		70% ALCOHOL-SOLUBLE N		70% ALCOHOL-INSOLUBLE N	
	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K
	First harvest (24-day-old plants)							
Leaves	27.8	29.8	6.42	6.55	0.99	1.21	5.43	5.33
Upper stems	43.1	43.8	4.30	4.92	2.51	3.22	1.79	1.70
Middle stems	38.4	40.3	3.58	4.46	2.49	3.47	1.09	0.99
Lower stems	43.8	33.0	3.80	3.59	2.92	2.60	0.88	0.99
	Second harvest (31-day-old plants)							
Leaves	29.4	28.9	6.08	6.59	1.21	1.27	4.87	5.31
Upper stems	36.4	41.9	3.81	4.87	2.16	3.26	1.65	1.60
Middle stems	34.1	38.8	3.57	4.29	2.64	3.44	0.93	0.85
Lower stems	29.5	38.5	3.43	3.75	2.57	3.06	0.86	0.68
	Third harvest (42-day-old plants)							
Leaves	28.6	29.4	5.72	5.81	0.70	0.83	5.03	4.97
Upper stems	29.8	44.7	2.94	3.09	1.40	2.08	1.54	1.01
Middle stems	27.6	36.4	3.55	2.84	2.64	2.16	0.91	0.68
Lower stems	25.9	30.2	3.58	2.49	2.77	1.92	0.81	0.57

stems having the highest concentration.

Potassium-starved leaves were higher at each harvest in total sugars and reducing sugars than complete-nutrient ones (table 4). This was also true of stems of the first harvest, but, as the deficiency developed, differences were smaller and at the third harvest were completely reversed owing to the low reducing-sugar content of minus-potassium stems. Sucrose was consistently higher in minus-calculated as percentage of total sugars was higher in no-potassium than in complete-nutrient leaves at the third harvest only. Starch concentrations were low in both groups of plants. Differences were small and not consistent. All the carbohydrates were higher in stems than in leaves of plus-potassium plants. This was true of minus-potassium plants of the first harvest but not consistently so at later harvests. Total-carbohydrate per-

centages, calculated for tops of potassium-starved and complete-nutrient plants, were, for the first harvest, 7.54 and 4.54, respectively; for the second, 6.13 and 3.89; and for the third, 4.98 and 10.24. The corresponding percentages for whole stems were 9.21 and 6.24 for the first harvest; 5.20 and 5.57 for the second; and 4.35 and 14.20 for the third.

TABLE 3

AMMONIA, AMIDES, AND NITRATES (PERCENTAGES ON DRY-WEIGHT BASIS)

PLANT PART	AMMONIA N		AMIDE N		NITRATE N	
	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K
	First harvest (24-day-old plants)					
Leaves	0.004	0.000	0.036	0.018	0.418	0.820
Upper stems	.025	.000	.042	.023	1.994	2.817
Middle stems	.016	.000	.020	.006	2.176	3.026
Lower stems	0.004	0.000	0.040	0.020	2.637	2.303
	Second harvest (31-day-old plants)					
Leaves	0.060	0.000	0.051	0.014	0.411	0.678
Upper stems	.059	.034	.038	.000	1.628	2.730
Middle stems	.038	.012	.010	.000	2.247	3.191
Lower stems	0.025	0.000	0.048	0.000	2.413	2.929
	Third harvest (42-day-old plants)					
Leaves	0.096	0.000	0.055	0.028	0.331	0.328
Upper stems	.059	.021	.043	.046	0.917	1.754
Middle stems	.041	.016	.053	.011	2.194	1.911
Lower stems	0.020	0.009	0.025	0.007	2.423	1.759

POTASSIUM.—Potassium tests were qualitative, but, since the solutions tested were five times as concentrated as the original extracts, rather accurate comparisons were possible.

Minus-potassium leaves contained only from one-half to one-eighth as much potassium as plus-potassium leaves, the differences being greater at the second and third harvests. Differences between upper stems of the two groups were even greater than in the leaves. At the first harvest potassium was found throughout potassium-starved stems, although much more at the upper level. At the second and third harvests, however, it was detected at the upper level only; and at the third harvest and upper level the test, although definite, was rather faint. Minus-potassium leaves contained two to five times as much potassium as the corresponding upper stems. In plus-potassium plants, however, upper stems contained two to four times as much of the element as the leaves, and middle and lower stems were even higher in potassium than the upper segments. Although the tissue had been extracted seven times with 70% alcohol after pouring off the

preserving alcohol, a small amount of potassium was still found in the finely ground complete-nutrient stems and in the leaves of each set of plants. The test on potassium-starved stems was inconclusive. None could be detected with sodium cobaltinitrite, but the platinum chloride test indicated a very small amount present.

stunting, and somewhat lower water content in comparison with plants receiving complete nutrient. The greener color of the leaves was noticed before chlorosis and before much, if any, stunting had occurred.

TABLE 4

CARBOHYDRATE FRACTIONS (PERCENTAGES ON DRY-WEIGHT BASIS)

Plant part	Total sugars		Reducing sugars		Sucrose		Sucrose percentage of total sugars		Starch	
	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K	Minus K	Plus K
	First harvest (24-day-old plants)									
Leaves	4.39	1.34	3.57	1.01	0.82	0.33	18.67	24.75	0.52	0.52
Upper stems	12.65	8.48	10.87	7.49	1.78	.99	14.06	11.65	1.22	1.17
Middle stems	7.44	4.79	5.55	4.04	1.89	.75	25.39	15.60	0.85	0.84
Lower stems	4.01	2.83	2.81	2.04	1.20	0.79	29.98	27.92	0.68	0.84
	Second harvest (31-day-old plants)									
Leaves	6.84	1.23	4.41	0.77	2.43	0.47	35.47	37.81	0.67	0.41
Upper stems	6.56	7.78	4.34	6.61	2.22	1.17	33.86	15.04	1.01	1.09
Middle stems	4.22	3.60	2.73	2.71	1.50	0.88	35.42	24.54	0.74	0.77
Lower stems	2.08	2.65	1.12	1.80	0.96	0.85	46.26	32.13	0.67	0.66
	Third harvest (42-day-old plants)									
Leaves	5.24	3.48	3.50	2.94	1.74	0.55	33.23	15.74	0.59	0.51
Upper stems	5.84	21.23	3.23	20.11	2.61	1.12	44.61	5.26	.82	.82
Middle stems	3.02	12.65	1.44	11.83	1.58	0.82	52.44	6.46	.53	.61
Lower stems	1.56	6.92	0.83	6.24	0.74	0.68	47.12	9.88	0.56	0.56

Discussion

Deficiency symptoms

Main potassium-deficiency symptoms in the sunflower plant were the deeper-green color of leaves, chlorosis of lower leaves followed by necrosis, general

These symptoms correspond essentially to those described in the literature (**7, 11, 12, 18, 21**). A number of investigators have emphasized the dark-green leaf color (**1, 16, 18**). McMurtrey (**18**) stated that this was the first symptom observed in the tobacco plant and that the green color had a bluish cast. The bluish tinge was not observed in the present work except in extremely stunted plants grown without potassium from planting.

In these it was evident, especially in the cotyledons.

Two distinct types of growth resulting from potassium deficiency have been observed in the tomato plant (**21, 29, 30**). NIGHTINGALE (**21**) found that after 3 weeks minus-potassium plants resembled those on minus-nitrogen. Leaves were chlorotic, and stems were hard and woody, with thick-walled pericyclic fibers and a high proportion of thick-walled xylem and collenchyma tissue as compared with plus-potassium plants. This type of growth was correlated with a high carbohydrate content. Later, the potassium-starved plants changed decidedly in appearance. Leaves which were chlorotic became dark green, and later-formed leaves were dark green. Although portions of the stem already formed retained their hard woody character, the new growth was soft, with thin-walled pericyclic cells and little xylem or collenchyma. Much of the xylem present was parenchymatous. A carbohydrate content not markedly different from that of the complete-nutrient plants was correlated with this type of growth.

These two growth habits were not observed in the sunflower plant, although the carbohydrate content of minus-potassium plants was quite low late in the deficiency. At the third harvest these plants were much lower in carbohydrates than were those receiving potassium.

EFFECTS OF POTASSIUM DEFICIENCY ON CHEMICAL CONSTITUTION

NITROGEN FRACTIONS.—The main effect of potassium deficiency on the nitrogen fractions was the accumulation of ammonia and amides in leaves and stems. Amino acids, though not determined, presumably also accumulated, since concentrations of these two groups of compounds usually run more or less parallel. These results correspond essentially with much reported data (**10, 13, 21, 26, 31**), although accumulation was not so pronounced as has been found in other plants. On the other hand, PHILLIPS *et al.* (**23**) could find no evidence of deranged nitrogen metabolism in the tomato plant at an early stage of potassium deficiency.

There are two main explanations in the literature for the high content in potassium-deficient plants of soluble organic nitrogen fractions. WALL (**31**) concluded that the accumulation of amino acids and amides in tomato plants at an early stage of potassium deficiency was a consequence of the need for potassium in the condensation of amino acids to proteins. Evidence for this viewpoint was the accumulation of carbohydrates along with the amino acids and amides and the low protein content of the plants. The only way to account for the high carbohydrate content was by some interference with protein synthesis. Interference at the amino acid stage would account for the accumulation both of soluble organic nitrogen fractions and of carbohydrates. The derangement could not have been at the nitrate-reduction stage, since nitrates did not accumulate. Further, interruption of protein synthesis at this stage would not account for the accumulation of amino acids and amides. WALL suggested (**29**) that potassium may act as a catalyst in the condensation of amino acids to proteins.

On the other hand, RICHARDS and TEMPLEMAN (**26**) concluded that potassium is not involved in protein synthesis in plants but that it is needed for maintenance of the protoplasmic complex. In its absence protoplasm breaks down, and proteins are hydrolyzed. They considered that proteolysis accounts for the accumulation of soluble organic nitrogen fractions in potassium-deficient plants. In

the work of WALL (**31**) these fractions accumulated in tomato plants early in potassium deficiency, when the carbohydrate content of the plants was high. He stated that proteolysis could not have accounted for their accumulation at this stage. The process does not go on rapidly when there is an abundance of carbohydrates. He admitted that proteolysis undoubtedly occurred late in the deficiency, when carbohydrates decreased and amino acids and amides increased rapidly.

Although the conclusion of RICHARDS and TEMPLEMAN (**26**) was a general one, their analytical data were derived from the study of barley leaves. GREGORY and co-workers (**8, 10**) found that potassium deficiency lowered the carbohydrate content of barley leaves. They did not find the initial high-carbohydrate phase as reported by WALL (**31**) and other investigators. There are two possible explanations of the difference between the viewpoint of WALL and that of RICHARDS and TEMPLEMAN. Potassium deficiency may have a different effect on barley than on tomato. Barley leaves may not have a high carbohydrate content early in the deficiency. In this case proteolysis might be the main factor accounting for the accumulation of soluble organic nitrogen fractions. However, if the high-carbohydrate phase is short in barley leaves, analyses may have been made too late to detect it. In this case both of WALL's explanations, (*a*) interference with protein synthesis and (*b*) proteolysis, might be needed to account adequately for the high content of the soluble nitrogen fractions in the leaves, the first applying early in the deficiency, and the second later. HARTT (**13**) reached a similar conclusion in her work on potassium deficiency of sugar cane. There was no accumulation of carbohydrates. She suggested that either the high-carbohydrate phase did not occur in sugar cane or that the analyses were made too late to detect it.

The carbohydrate and nitrogen relation in the sunflower plants of this study resembled that found by WALL (**31**) in the tomato. It would seem that both proteolysis and the need for potassium in protein synthesis were factors accounting for the accumulation of soluble organic nitrogen fractions in potassium-starved sunflower plants, the second being the dominant factor early in the deficiency and proteolysis later.

CARBOHYDRATE FRACTIONS.—Many investigators have found that carbohydrates accumulate in potassium-deficient plants in comparison with those on complete nutrient (**16, 21, 27, 31**). On the other hand, others have found no accumulation (**8, 10, 13**), and GREGORY and co-workers (**8, 10**) noted that potassium starvation lowered the carbohydrate content of barley leaves. The carbohydrate situation, at least in some potassium-deficient plants, seems to depend on the stage of the deficiency. NIGHTINGALE (**21**) and WALL (**29, 30**) found two distinct stages in the type of growth of potassium-starved tomato plants, an early one characterized by chlorosis of leaves and by hard woody stems, and a later stage in which the leaves were green and the new growth of the stems was soft. The first stage was correlated with a high carbohydrate content, the second with a relatively low one.

The carbohydrate situation in the sunflower in this study resembled essentially that found by NIGHTINGALE (**21**) and WALL (**29**) in the tomato, although the sunflower did not show the two types of growth of the tomato. WALL found that the high-carbohydrate phase of potassium-deficiency in the tomato resulted

from an accumulation of hexoses, sucrose, and starch and that all these carbohydrates decreased at later stages of the deficiency. In the present work hexoses and sucrose accumulated early in potassium starvation, and the low carbohydrate content of the sunflower plants later in the deficiency resulted from a decrease in reducing sugars. Sucrose remained high in minus-potassium plants in comparison with plus-potassium ones to the end of the experiment. Starch content did not differ much in the two sets of plants at any time.

NIGHTINGALE (**21**) explained the initial accumulation of carbohydrates in minus-potassium tomato plants as due to the interruption of protein synthesis at the nitrate-reduction stage. In this case nitrates should also have accumulated; but the minus- and plus-potassium plants had about the same nitrate content. This was explained by the large amount of nitrate in the plants of each set. When plants of each series, both equally high in nitrates, were grown without a nitrate supply, the plants on complete nutrient soon exhausted their tissues of nitrate, but the potassium-starved ones remained high in nitrate until death. But WALL (**29**) pointed out that, if absorption continued, this retention of nitrates could be explained as due to interference with protein synthesis at another stage. Since in his own work both carbohydrates and soluble organic nitrogen compounds accumulated in potassium-deficient tomato plants, while nitrates did not, he concluded that nitrate reduction was not interrupted but that potassium was necessary for the condensation of amino acids to proteins. Interference at the nitrate-reduction stage would account for accumulation of carbohydrates but not of amino acids and amides.

Carbohydrates would not seem to be necessary for the condensation of amino acids to proteins. However, whether amino acids result from the hydrolysis of proteins, as in germinating seeds, or from the reduction of nitrates, they may be changed to amides and then the amides used in protein synthesis. There is much work indicating that carbohydrates are needed for the synthesis of proteins from amides (**2, 20**). STEWARD and STREET (**28**) concluded that protein synthesis in potato disks was canalized through the amide, glutamine. They considered that ammonia for the synthesis of glutamine came from amino acids, asparagine, or reduction of nitrates. Perhaps the interruption of protein synthesis in the experiments of WALL (**31**) was at the amide stage.

WALL (**31**) regarded the fall in carbohydrate content of potassium-starved tomato plants to be due to decreased photosynthesis and increased respiration. Several investigators (**6, 9, 17, 24**) have found that photosynthesis is lower in potassium-starved plants than in those on complete nutrient. WALL (**31**) stated that potassium may have either a direct or an indirect effect on the process of photosynthesis. Photosynthesis may be lower in potassium-deficient plants owing to the poor condition of the protoplasm resulting from deranged nitrogen metabolism. PIRSON (**24**) found that photosynthesis was low in potassium-starved *Chlorella* cells. The addition of potassium brought about recovery in two distinct phases. At first the potassium ion directly effected the increased rate of photosynthesis. Then there was a secondary rise associated with formation of chlorophyll and other cell materials. GREGORY and co-workers (**9, 10, 25**) found that respiration was higher in potassium-starved barley leaves. With decreased

photosynthesis and increased respiration the carbohydrate content of plants would be expected to be low.

The initial high carbohydrate content of minus-potassium sunflower plants of this study probably partly resulted from interference with protein synthesis at the amide stage. Accumulation of amides (and presumably of amino acids), however, was quite low—too low, it would seem, to account entirely for the rather high carbohydrate content of potassium-starved plants in comparison with those on complete nutrient. Another factor, probably of considerable importance, was the larger leaf-stem ratio of the former plants. At the first harvest this ratio was 1.063 for minus-potassium plants and 0.726 for plus-potassium ones. Accordingly, potassium-starved plants had a greater photosynthetic leaf area in proportion to the weight of their stems than did those on complete nutrient and would be expected to be high in carbohydrates. The decrease in carbohydrates of the sunflower plants at late stages of potassium deficiency probably resulted from decreased photosynthesis and increased respiration.

POTASSIUM.—Potassium is found in particular abundance in meristematic tissue of potassium-starved plants (**16, 21**). In no-potassium tomato plants NIGHTINGALE (**21**) found the element definitely localized in stem and root tips, in developing abscission layers, and in cambium. There was enough potassium for development of the stem tip but not sufficient for cambial activity in older portions of the stem so that there was considerable increase in length of stem but little in diameter.

Most of the potassium in plants is water soluble (**16, 19, 21**). MORRIS and SAYRE (**19**) found that, with the possible exception of that in the cob, all the potassium in corn tissue was water soluble and could be determined with equal accuracy by analyzing an extract, the expressed juice, or the dried tissue. On the other hand, OLSEN (**22**) noted that in the summer months about 30% of the potassium of frozen beech leaves, and presumably of the intact tissue, was adsorptively bound. The element was removed entirely by continued washing with water or by treating the tissue with 0.1 *N* calcium chloride solution. The amount of adsorptively bound potassium decreased late in the season, and shortly before defoliation all the element was dissolved in the cell sap.

Potassium is a mobile ion. In potassium-deficient plants it moves to regions where needed, particularly from older tissues to meristematic ones (**16, 21**). In potato plants on complete nutrient JAMES (**15**) found that the element moved from one organ to another either with or against the concentration gradient.

Results of the present study are in accord with the literature discussed above. In minus-potassium sunflower stems, late in the deficiency, potassium could be detected only at the upper level. In complete-nutrient stems throughout the experiment the element was found in all parts, the concentration being even higher at the middle and lower levels than at the upper. Although most of the potassium was removed by seven extractions with 70% alcohol, a small amount was still present in the plus-potassium stem residues and in the leaf residues of each series. The test on the minus-potassium upper stems was indefinite. None could be detected by the sodium cobaltinitrite reagent, but by the platinum chloride test a very small amount seemed to be present.

No specific translocation studies were

made. As mentioned above, late in the deficiency potassium was restricted to the upper level of potassium-starved stems but throughout the experiment had a general distribution in those with complete nutrient. This would indicate that, in the former stems during development of the deficiency, potassium was translocated from lower to upper stem levels. An experiment in which sunflower plants were grown from seed to maturity, a period of 66 days, without potassium also indicated movement of the element from lower to the upper parts of the plants. Although some plants died, others did develop small flower heads. Since potassium seems to be necessary for cell division (**16, 21**), development of these heads indicated movement of the element into plant tops from the lower leaves and levels of the stem.

Summary

1. Effects of potassium deficiency were studied in sunflower plants grown in sand cultures. The first symptom observed was a deeper-green color of the leaves. Later, lower leaves became chlorotic, the decrease in chlorophyll starting at the tips and edges and then extending inward and toward the base. Chlorosis was followed by necrosis of the tissue. Minus-potassium plants were stunted, with small leaves and short, thin stems. No difference in hardness of stems of the two sets of plants was noticed. Potassium deficiency affected tops more than roots, and growth of stems was limited more than that of leaves. Thus, top-root ratios of minus-potassium plants were lower and leaf-stem ratios were higher than those of plus-potassium plants.

2. Potassium-starved leaves and stems tended to be lower in water content than those with complete nutrient. In general, total nitrogen, soluble nitrogen, and nitrates were higher in leaves and stems of the latter than of the former. In the middle and lower stems of the third harvest, however, both total nitrogen and soluble nitrogen were higher in the former plants. Ammonia and amides accumulated in minus-potassium leaves and stems, although the amide percentages were rather low. Their accumulation at early stages in the deficiency may have resulted mainly from interference with protein synthesis at the amide stage; at late stages, proteolysis was probably an additional important factor.

3. Total sugars and reducing sugars were high in minus-potassium leaves throughout the experiment. This was true also of stems of the first harvest, but differences lessened as the deficiency developed and at the third harvest were in favor of the plus-potassium stems, owing to the low reducing sugar content of potassium-starved stems. Sucrose was uniformly higher in minus-potassium leaves and stems than in plus-potassium ones. Starch did not vary much in the two groups of plants. When all carbohydrates were included and calculated for either tops or stems, the no-potassium plants were high in carbohydrates in comparison with those on complete nutrient at the first harvest but low at the final harvest. Accumulation of carbohydrates early in the deficiency may have resulted both from the interference with protein synthesis at the amide stage and from the greater leaf-stem ratio. The low carbohydrate content at late stages may have been a consequence of reduced photosynthesis and increased respiration.

4. At the final harvest potassium could be detected only in leaves and upper stems of the minus-potassium plants. It was well distributed throughout tops of the plus-potassium plants at all times,

being even more concentrated in middle and lower stems than in upper. Most of the potassium in the leaves and stems of each set of plants was removed by seven extractions with 70% alcohol.

Potassium seemed to be readily translocated in potassium-starved plants as indicated by its differential distribution in the two sets of stems and by the fact that plants grown for 66 days with a minus-potassium solution developed small flower heads.

The writer wishes to express his appreciation to Professor J. M. Beal for his interest and many helpful suggestions and for making the photographs used as accompanying illustrations.

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TRANSLOCATION OF RADIOACTIVE OCTAMETHYL PYROPHOSPHORAMIDE IN BLACK VALENTINE BEAN PLANTS[1]

RANDOLPH T. WEDDING AND ROBERT L. METCALF

Introduction

The recent discovery of the so-called systemic insecticides offers great potentialities for a completely different approach to the problem of insect control on both ornamental and crop plants. In their original form certain of these materials may have little or no toxicity for insects, but, when applied on a portion of a plant or to soil containing the roots, they are distributed to all parts of the plant in an altered form and cause the death of insects feeding on any part.

It is obvious that new methods of utilizing such materials in pest control are possible. Since a small amount of the insecticide applied on a portion of the leaves enters the plant and is distributed throughout, thus giving the entire plant protection against insects, the need for a full-coverage spray is eliminated. It is also possible to place the insecticide in or on the soil or in irrigation water or to treat seeds at time of planting and thus afford protection for the developing plant.

The systemic properties of octamethyl pyrophosphoramide, which is also known as bis-(bis-dimethylamino)phosphonous anhydride, or OMPA, were established by KÜKENTHAL (**7, 13**) shortly after its synthesis by SCHRADER (**12**) and have been confirmed by subsequent investigators (**2, 3, 6, 10, 11, 14**).

To understand the way in which these systemic insecticides differ from other insecticides, and to provide a sound basis for developing field uses of the materials, it is necessary to obtain information regarding their distribution in the plant. Data on velocity of movement, tissues in which the material accumulates, and the effect of various environmental factors on its distribution and accumulation are needed. The present study is concerned with the movement and accumulation of OMPA in bean plants, using a specially

[1] Paper no. 738, University of California Citrus Experiment Station, Riverside, California. This investigation was financed in part by a grant from the U.S. Atomic Energy Commission, Contract AT (11-1) 34, Project 6; Dr. F. M. TURRELL, Director.

synthesized sample of the compound labeled with radioactive phosphorus (P^{32}).[2]

Material and methods

The bush bean *Phaseolus vulgaris* variety Black Valentine, was used in these studies. Plants were grown in the greenhouse, four to an 8-inch pot, in a standard potting medium. Three to 4 weeks after emergence, when the second trifoliolate leaf was beginning to unfold, plants were selected for uniformity, and 100 ml. of a solution containing 10 mg. of P^{32}-labeled OMPA diluted with 90 mg. of the nonradioactive form of the insecticide were applied to the soil in each pot.

In the first experiment, harvesting was started 2 hours after application and continued at intervals for 120 hours. All data given are the means of values for the two plants harvested at each time of collection. When harvested, each plant was divided into six sections, as follows: (I) stem from soil to primary leaf, (II) stem from primary leaf to first trifoliolate leaf, (III) stem from first to second trifoliolate leaf, (IV) primary leaves, (V) first trifoliolate leaf, and (VI) second trifoliolate leaf (leaf plus terminal bud), as indicated in figures 1–6. These section samples were dried at 70° C. in a mechanical convection oven, weighed, and then ashed separately in a muffle furnace at a temperature of 500° C. The ash was spread uniformly over the surface of an aluminum planchet, and the activity of P^{32} in the ash was determined by means of a thin-walled, end-window Geiger-Müller tube and scaling unit. After correction for background counts, the activities found were converted to micrograms of OMPA by equating with the activity of a standard sample of the material counted at the same time. The decay rate determined for a carefully measured amount of the P^{32}-labeled OMPA thus corrects for the decay of P^{32} in the plant tissue.

In the second experiment, determinations were made successively on living leaves of the same plant. The activity of P^{32} was measured by placing a thin-walled, end-window Geiger-Müller tube in contact with the leaf and estimating the activity by means of a counting rate meter.

In a third experiment to determine the activity of materials extractable from the tissue, samples were taken from plants treated with OMPA by cutting 0.2 gm. of blade material from a total of three leaves per plant. Each sample was homogenized in 3 ml. of distilled water. Then 1 ml. of 3 *N* NaOH was added to 2 ml. of the homogenate, the samples were shaken for 1 minute, and 3 ml. of chloroform were added. The samples were again shaken for 1 minute and centrifuged to separate the layers. One milliliter of each layer was then wet-ashed with nitric acid at 300° C. to determine the partition of radioactivity.

Cholinesterase activity of the leaf *brei* was measured manometrically by adding 0.1 ml. of the homogenate to 2.6 ml. of buffer containing 0.025 *M* $NaHCO_3$, 0.15 *M* NaCl, 0.04 *M* $MgCl_2$, and three homogenized housefly heads (brains used as cholinesterase source) per milliliter (**9**) in a Warburg flask. The side arm contained 0.3 ml. of 0.1 *M* acetyl choline bromide. The flasks were equilibrated at 37° C. with 5% CO_2 and 95% N_2 for 5 minutes, then tipped, and the *brei* and substrate were mixed for 5 minutes. To establish the percentage of inhibition, the amount of CO_2 (in microliters) produced in 30 minutes was then compared with that of a standard containing *brei* from untreated beans.

[2] Supplied through the courtesy of Pest Control, Ltd., Cambridge, England.

Bio-assays with the two-spotted mite, *Tetranychus bimaculatus* Harvey, were made on the remainder of the leaves used for radio-assay by placing these in Munger cells (**8**) and infesting them with twenty-five adult female mites per cell. Mortality counts were made in 24 and 48 hours.

Results

The concentration of OMPA in micrograms per gram of dry weight as a function of elapsed time for each of the six sections of the plants of the first experiment is shown in figures 1–6. The concentration of the insecticide in the lowest section of the stem ascends rapidly at first, then levels off, and appears to turn downward (fig. 1). This was the oldest portion of the stem tissue and was probably fully expanded, since no growth was apparent during the period of the experiment. The curve for this tissue has the same general shape as the curves for the other stem tissues (figs. 3, 5), which were younger and still actively growing, as was evident by their elongation during the experiment; but the maximum value is lower for this tissue than for the others. The activity curves for the younger tissues also show a more rapid initial rise than those for the older (lower) tissues of the stem. Both of these facts seem to indicate that either OMPA or some breakdown product of OMPA was being accumulated by the younger, more actively metabolizing tissues. The reason for the apparent downward trend in the curve for the lowest stem section is not immediately apparent, but it may be that the high point in the curve is at the time when a maximum amount of the P^{32}-labeled material was moving through the stem and that the downward slope of the curve was due to a diminution in the amount of mobile P^{32} passing through this section, with the lower value approaching that for the P^{32}-labeled material which was fixed in the tissues.

Figures 2, 4, and 6 show the build-up of OMPA in the leaves. The accumulation of activity in the primary leaves was slower than in the trifoliolate leaves but eventually reached as high a concentration. It is particularly noticeable that in none of the leaves did the concentration ascend as steeply as in the stems. In all the leaves except the primaries the concentration tended to level off before the end of 120 hours. The more rapid uptake of the insecticide by the younger leaves followed the same tendency as that apparent in the stems. This is in agreement with the results of RIPPER *et al.* (**10, 11**), which showed that the chemical was taken to areas of new growth and that it was generally more effective in young plants than in mature ones.

These data make it possible to calculate the approximate velocity of the translocation of OMPA or its breakdown products through the stem during the first 24 hours of the experiment, when the concentration of OMPA in the plants was rising rapidly. For this purpose a number of assumptions and approximations must be made. The velocity of transport of the P^{32}-labeled material past the insertion of the primary leaves at the top of plant section I is calculated by the following equation:

$$V = \frac{\left[\sum_{n=\mathrm{II}}^{n=\mathrm{VI}} A_n\right]_{t_2} - \left[\sum_{n=\mathrm{II}}^{n=\mathrm{VI}} A_n\right]_{t_1}}{F A_{\mathrm{I}} \dfrac{(t_2 - t_1)}{L}},$$

where V = velocity in cm/hr; A = μg. OMPA per tissue section, calculated from P^{32} determinations; F = fraction of OMPA in stem section which is assumed to be mobile; t = time of harvest; L = length of section I from soil line to pri-

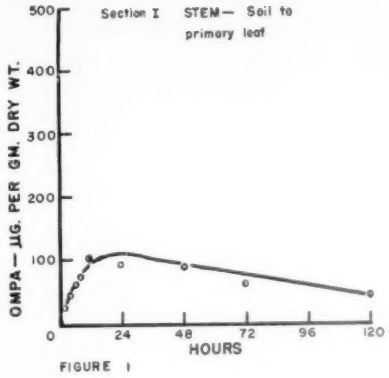

FIGURE 1

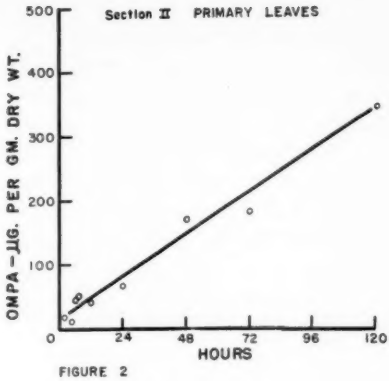

FIGURE 2

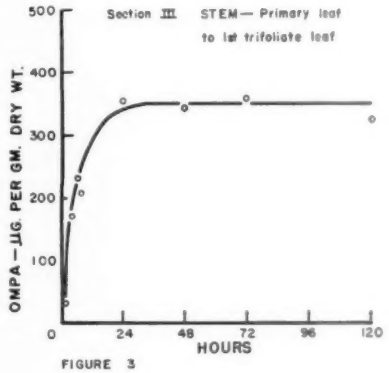

FIGURE 3

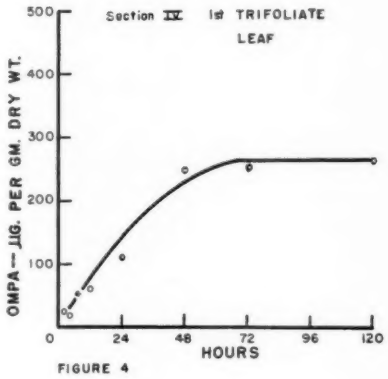

FIGURE 4

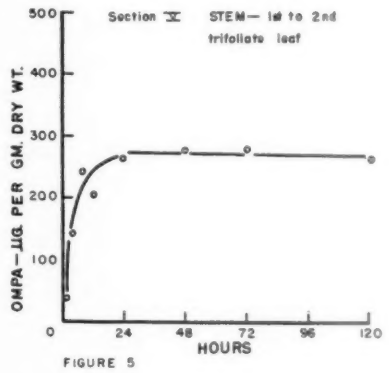

FIGURE 5

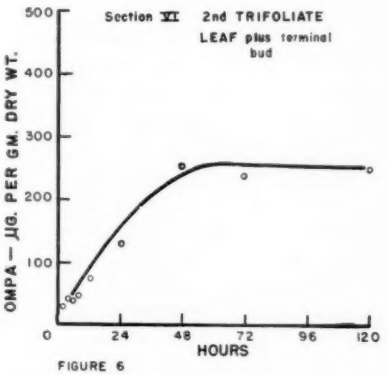

FIGURE 6

FIGS. 1–6.—Uptake of octamethyl pyrophosphoramide (OMPA) by Black Valentine bean plants over a period of 120 hours after soil treatment, as shown by concentration (μg/gm dry weight) in plant samples (secs. I–VI).

mary leaves; and n = plant section (I, II, etc.).

This calculation is based on the assumption that all the OMPA in the top of the plant moved past the top of section I at a constant rate between the harvest at time t_1 and that at time t_2 and that only half of the OMPA found in section I at a given time was mobile, the remainder being fixed in the cells. This last assumption is based on the fact that the concentration in section I at the end of 120 hours was about half of that in the same section at the maximum. This does not indicate, of course, that the same relationship held during the first 24 hours of the experiment, when the concentration of OMPA in all parts of the plant was increasing rapidly. Different values substituted in the equation for this assumption will materially affect the calculated velocity. For example, if it is assumed that only one-fourth of the OMPA found in section I was mobile, the calculated velocity would be twice as great as that based on the assumption that half of it was mobile.

By use of this equation and by calculating rates of movement for different periods of time when the concentration of OMPA in the plant was increasing rapidly, rates ranging from as low as 17 cm/hr to as high as 58 cm/hr were found. Most values were of the order of 20 cm/hr, and this is thought to represent the best approximation of the rate of movement of OMPA through the bean stem in this experiment.

In an attempt to eliminate the variability resulting from the use of different plants for successive determinations of OMP^{32}A content of the tissue, determinations (figs. 7–10) were made on living leaves of the same plants by placing a thin-walled, end-window Geiger-Müller tube in contact with the leaf. Each point on the curves represents the mean of determinations made on four plants. The activity values are merely counts per minute, a relative figure, and give no information regarding absolute amounts of OMPA. It should also be noted that these counts-per-minute values are for a constant area of the leaf tissue—the area of the Geiger-Müller tube window. Thus the total amounts of OMPA in the various leaves may be somewhat different from that shown, but the values do represent a relative concentration per unit area of leaf tissue.

The data obtained in this experiment show the same trend as those obtained previously. The slope of the concentration curve for the primary leaves is not so steep as that for any of the trifoliolate leaves. This indicates that OMPA probably builds up first in the younger leaves and then accumulates more slowly in the older leaves. This experiment was concluded after 72 hours, and in that time none of the leaves showed the leveling-off in concentration found in the leaves of the previous experiment (figs. 2, 4, 6).

The correlation of radioactive material extractable from the leaf with the mortality of the two-spotted mite and with the inhibition of cholinesterase activity is shown by the data given in table 1. The fractionation of the total radioactivity between chloroform and aqueous sodium hydroxide by the method of RIPPER *et al.* (11) separates the chloroform-soluble, unchanged OMPA from breakdown products that are taken up by the alkali layer. The total concentration of OMPA rose constantly up to 8 days, and at that time detectable amounts of radioactive phosphorus appeared in the alkali-soluble fraction.

The 48-hour mortality of the two-spotted mite feeding on tissues from the OMPA-treated plants increased during

the experiment and after 8 days approached 100%. The amount of cholinesterase inhibitor formed was variable, and the data indicate that it did not accumulate in the leaves as did the unchanged OMPA. Apparently the inhibitor is unstable in living plant tissues and is rapidly destroyed (4).

Discussion

From the experiment in which Black Valentine bean plants were divided into six sections and the distribution of $OMP^{32}A$ was determined by counts made on the tissue ash, data were obtained bearing on the question of the tissues in which the insecticide accumulates. The

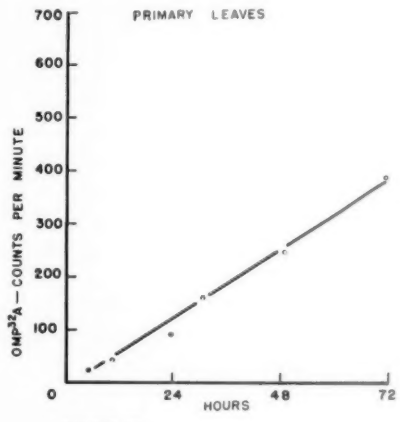

FIGURE 7

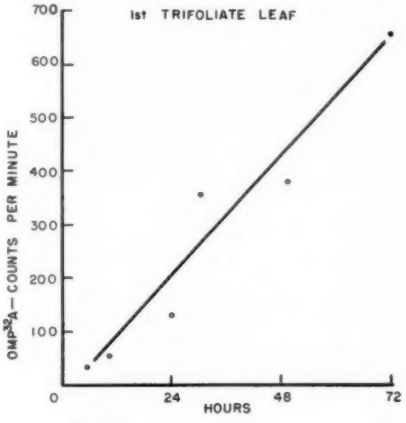

FIGURE 8

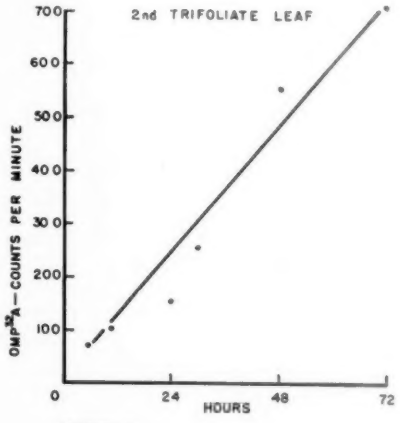

FIGURE 9

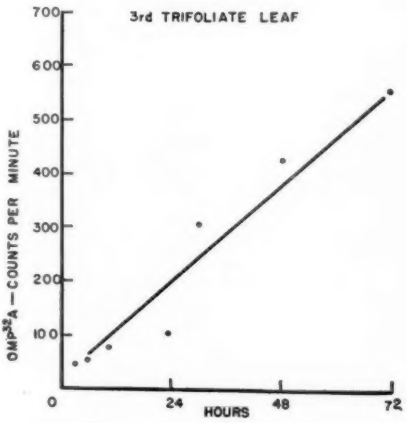

FIGURE 10

FIGS. 7–10.—Relative activity of $OMP^{32}A$ in four living leaves of Black Valentine bean plants over a period of 72 hours after soil treatment.

tendency was for those tissues which were physiologically younger to show a more rapid build-up of OMPA concentration. This was true both in stem and in leaf tissues, but the concentration rose more rapidly in the stem than in the leaves. One reason for this might be that the OMPA must pass through the stem to reach the leaves, but that this was not the only factor involved is indicated by the fact that all sections of the plant above the lowest stem section contained a higher final concentration than this one, through which all the OMPA must have passed. It seems more probable that the OMPA, or at least the P^{32} in some form, was accumulated to a greater extent by the younger, less differentiated cells of the stem.

Another feature of the curves from the first experiment is the tendency for the concentration of OMPA to level off or to become stable during the time of the experiment in all tissues except the primary leaves. The probable explanation of the flattening of the curves for the trifoliolate leaves is that the leaf dry weight was increasing at the same time that the amount of OMPA in the leaves was rising. That this was happening is indicated by the tendency of the trifoliolate leaves to increase in dry weight during the experiment and by the more or less steady increase in absolute values of OMPA (μg/leaf) in these leaves at this same time (table 2). The stem sections and the primary leaves showed no such steady increase in dry weight. Thus it appears that the leaf tissues differed from the stem tissues in that, although the concentration of OMPA built up more slowly in the leaf tissues, their capacity for fixing OMPA was greater than that of the stem tissues, and a longer time was required for them to reach the saturation point. GARDINER and KILBY (5), on the other hand, found that P^{32}-labeled OMPA accumulated more rapidly in the cotyledons of marrow beans than in the stem. After a greater lapse of time than occurred here, the con-

TABLE 1

CORRELATION OF RADIOACTIVE OCTAMETHYL PYROPHOSPHORAMIDE (OMPA) EXTRACTABLE FROM LEAVES OF SOIL-TREATED BLACK VALENTINE BEAN PLANTS WITH MORTALITY OF THE TWO-SPOTTED MITE AND WITH INHIBITION OF CHOLINESTERASE ACTIVITY*

DAYS AFTER SOIL TREATMENT	OMPA IN LEAVES (μG/GM FRESH WEIGHT)		PERCENTAGE MORTALITY OF TWO-SPOTTED MITES FEEDING ON LEAF TISSUES		PERCENTAGE INHIBITION OF CHOLINESTERASE ACTIVITY†
	Total	Alkali-soluble	After 24 hr.	After 48 hr.	
1	25	0	9	20	21
2	52	0	5	58	19
4	138	0	72	84	0
8	405	41	72	96	15

* Soil treated with 10 mg. $OMP^{32}A$ and 90 mg. OMPA in 100 ml. water. Four large leaves picked at given intervals for radio-assay and bio-assay.

† Cholinesterase from housefly brains.

TABLE 2

DRY WEIGHT AND TOTAL OMPA CONTENT OF FIRST AND SECOND TRIFOLIOLATE LEAVES OF BEANS HARVESTED AT INDICATED TIME AFTER TREATMENT*

HOURS AFTER TREATMENT	FIRST TRIFOLIOLATE LEAF (SECTION IV)		SECOND TRIFOLIOLATE LEAF (SECTION VI)	
	Dry weight (gm.)	OMPA (μg.)	Dry weight (gm.)	OMPA (μg.)
2	0.1264	3.16	0.1336	2.10
4	.1580	3.16	.0952	4.20
6	.1018	5.19	.1165	3.09
8	.....	.....	.1153	4.16
12	.0759	5.16	.1376	5.45
24	.1561	17.17	.1071	11.76
48	.0917	22.73	.1639	15.25
72	.1542	38.86	.2021	46.49
120	0.1887	49.05	0.2135	53.15

* Figures represent means of two separate determinations.

centration in the leaves would probably be higher than in the stem, despite the increased deposit of dry matter. After a further lapse of time the absolute amount of OMPA in the leaves might tend to level off because of decreased availability of the material from the soil and because of other factors such as the capacity of the leaves to contain and metabolize the insecticide.

The rate of movement of OMPA in the plant, as calculated from the data of this experiment, was of approximately the same order of magnitude as has been determined for the movement of organic materials of various types in the phloem (**1**). Since no separation of the movement of OMPA in the xylem and in the phloem was attempted here, however, nothing can be asserted as to the tissue in which the material moves. By use of bio-assay methods, ZEID and CUTKOMP (**14**) have recently found that OMPA applied to leaves moves more rapidly downward than upward; this indicates that movement is probably in the phloem.

The maximum recovery of OMPA from above-ground portions of the plants, which approached 1% of the total applied to the pot after 120 hours of exposure, was so low as to raise serious doubts regarding the economic advisability of using the material as an insecticide by soil application. It is probable that the percentage of recovery would be even less under field conditions, where the application could not be confined to a volume of soil so relatively limited as in the pots used here. This low efficiency might, of course, be improved in established plants, whose root density would be greater than that of the young bean seedlings of this experiment, but it seems likely that a partial-coverage spray application of OMPA would make a more efficient use of the systemic properties of this insecticide than would a soil application.

The second experiment, in which the activity of OMPA was determined by successive measurements on living leaves of the same plants, showed the same tendency for the trifoliolate leaves to contain more OMPA than the primary leaves after the same lapse of time and seems to confirm the finding that the more actively differentiating tissues have a greater ability to accumulate the material. This experiment showed no tendency for the concentration to level off before the end of 72 hours, probably because the time of exposure was less than in the first experiment. But since the measurements are on a unit-area basis rather than on a dry-weight basis, the straight lines probably indicate the same type of reaction as was shown by the absolute values of OMPA per leaf in the first experiment.

The in vivo determinations of this experiment lacked precision owing to (*a*) self-absorption of radiation from the P^{32} in the leaf tissue; (*b*) the possibility of an unequal distribution of OMPA in the leaf; and (*c*) the inherently low accuracy of the counting rate meter. These disadvantages were somewhat offset, however, by the fact that much of the biological variation of the plants was eliminated by making measurements successively on the same plants during the entire experiment.

The question whether the movement of OMPA as traced in this experiment by means of P^{32} determinations is actually a measure of the movement of the original compound introduced into the soil, or of some closely related but more toxic compound, or whether the measurements are actually of some breakdown product of OMPA or even of some inorganic com-

pound containing P^{32}, is resolved to a certain extent by the data given in table 1. These data indicate that no breakdown products were found until the eighth day, when some activity began to appear in the alkali-soluble fraction of the extract. RIPPER *et al.* (**11**) have found that pure OMPA is partitioned into chloroform from an aqueous extract but that inactive breakdown products are alkali-hydrolyzable and insoluble in chloroform. DUBOIS *et al.* (**4**) showed that OMPA itself is not the active insecticide in the plant but is changed to a compound related to OMPA within the plant and that this compound is actually the effective cholinesterase-inhibiting agent.

In addition to the data mentioned above, which indicate that the P^{32} remains in a form which acts as an insecticide, the mortality of the two-spotted mite feeding on leaves of plants treated with the $OMP^{32}A$ also showed that the material reaching the leaves was an active insecticide. The tendency of the percentage kill to become asymptotic with time probably did not result from a leveling-off of the insecticide concentration but rather from the fact that the kill was approaching 100%.

Summary

1. Octamethyl pyrophosphoramide, also known as bis-(bis-dimethylamino)-phosphonous anhydride, or OMPA, labeled with P^{32} was used in a study of its translocation in bean plants (*Phaseolus vulgaris*, Black Valentine var.) after uptake through the roots.

2. In both stem and leaf this material tended to accumulate more rapidly in younger than in older tissues.

3. After uptake from the soil the material moved through the stem at an approximate rate of 20 cm/hr.

4. Less than 1% of the material added to the soil was recovered from aboveground portions of the plant after 120 hours of exposure.

5. The P^{32}-containing compounds detected in the plant were toxic to insects, as determined by bio-assay. Insecticidally inactive breakdown products of OMPA were found after 8 days of exposure.

The authors wish to express their gratitude to Dr. S. J. RICHARDS for advice and to Miss MARGARET LARABEE for technical assistance.

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DESICCATION IN CONIFER LEAVES: ANATOMICAL CHANGES AND DETERMINATION OF THE LETHAL LEVEL

JOHNSON PARKER

Introduction

Previous work of the writer (**13**) demonstrated differences in moisture loss from excised leaves of different coniferous species. It was concluded that moisture retention above a certain level seemed essential to continued survival, thus indicating the existence of a lethal level of minimum moisture content.

The problem centers around determination of the point where leaves are "dead" in the course of dehydration and rehydration. Since natural color changes are often unreliable, a more precise measurement of the lethal level was sought. A compound believed to be an enzyme reactant was used previously, but there was doubt as to the validity of this test of viability (**13**). The present research therefore examined more closely the changes in conifer leaves occurring with desiccation to determine (*a*) whether the lethal level could be altered with experimental treatment, (*b*) whether the tetrazolium chloride test was a reliable measure of viability, and (*c*) the relation of released CO_2 to viability.

Material and methods

EXPERIMENT 1.—Leaves of white pine (*Pinus strobus* L.) and Austrian pine (*P. nigra* Arn., var. *austriaca* Asch. et Gr.) were dried in room air of 60% relative humidity and 20° C. Cross and longitudinal sections of the needles were made during desiccation and rehydration, and the sections were mounted in paraffin oil for microscopic examination.

EXPERIMENT 2.—Leaves of Austrian pine collected from the trees at about 0° C., brought to the laboratory in a thermos flask, and warmed to 17° C. within 4 hours, were dried in moving air that had passed through silica gel drier or in moving air that had bubbled through distilled water. Bases of needles were sealed in paraffin, since it was found that unsealed needles lost water more rapidly than sealed ones in the first 5 days of drying. Needles, when not in the measuring apparatus, were illuminated by incandescent light of 1000 foot-candles. Approximately every 24 hours about seventy fascicles of needles were placed in a glass tube (4 cm. in diameter

by 14 cm. in length) connected at both ends to an air stream which passed through plastic tubing to the constant-recording infrared gas-analyzer (**7**). The needles remained in the apparatus from 1 to 24 hours. The glass tube, which could be opened by means of a ground-glass joint, was kept in a thermos flask in the dark, and air from outside the building was warmed to 17° C. before passing over the needles. The difference between the CO_2 content of outside air and that which had passed over the needles was considered to be the amount released by them. The instrument was calibrated with nitrogen gas of known CO_2 content.

EXPERIMENT 3.—The rates of CO_2 release from Austrian pine needles which were being dried in the dark (*a*) in still air over a saturated NaCl solution or (*b*) in air over distilled water were compared. With the covers of the glass containers holding these solutions kept slightly open, relative humidities of about 70% and 95% were obtained. At various intervals in the experiment (fig. 5) the tetrazolium test was made on two pairs of needles from each treatment by the method previously described (**13**).

EXPERIMENT 4.—Various treatments were used in comparing rates of water loss in Austrian and white pine. Needles were dried on the laboratory table, and certain samples were rehydrated in distilled water at various intervals. After they reached their original weight, they were tested with tetrazolium chloride. Other samples were maintained at constant weight at moisture levels between 70% and 90% of their original weight by storage in closed containers over tap water. Then they were rehydrated in distilled water and tested as before. Still other samples were dried over saturated NaCl solutions, which give a relative humidity of 75.8% at 20° C. (**18**), or over anhydrous $CaCl_2$ in closed containers. Air was replaced daily to prevent accumulation of CO_2. Comparisons of drying rates between the same two species were also made in moving air of different moisture content as previously described. Cut ends of needles were sealed in paraffin.

EXPERIMENT 5.—Tetrazolium chloride tests were made on onion tissue subjected to different preliminary treatments. Scales, roots, and shoots were cooled to −14° C. within 5 hours and thawed at the same rate; other tissue was dried in air of 93% and 88% relative humidity for 5 days and rehydrated in air of 98% and finally 100% relative humidity. Still other tissue was boiled 2 minutes and compared with the others for evidence of a positive tetrazolium test. This test consisted of leaving the tissue in the dark for 12 hours in a 1% solution of tetrazolium chloride.

Results

EXPERIMENT 1.—When excised leaves of white and Austrian pine lose moisture and are unable to replace it, cells of the chlorenchyma, endodermis, and transfusion tissue decrease in size. One result of this shrinkage is that the epidermis and hypodermis bend inward (figs. 1, 2). In Austrian pine (fig. 1) the adaxial surface bends inward, and in white pine (fig. 2) all three surfaces bend inward. The condition of dehydrated leaves shown in figures 1 and 2 is only slightly above the level of moisture content which is lethal, provided the rate of drying is relatively rapid. If drying continues, the shrinkage may go even further than that illustrated, and the upper corners of the leaf, as seen in cross section in Austrian pine, bend in toward each other. Longitudinally the needles are commonly warped into a slight curve.

The change in shape of the cells inside

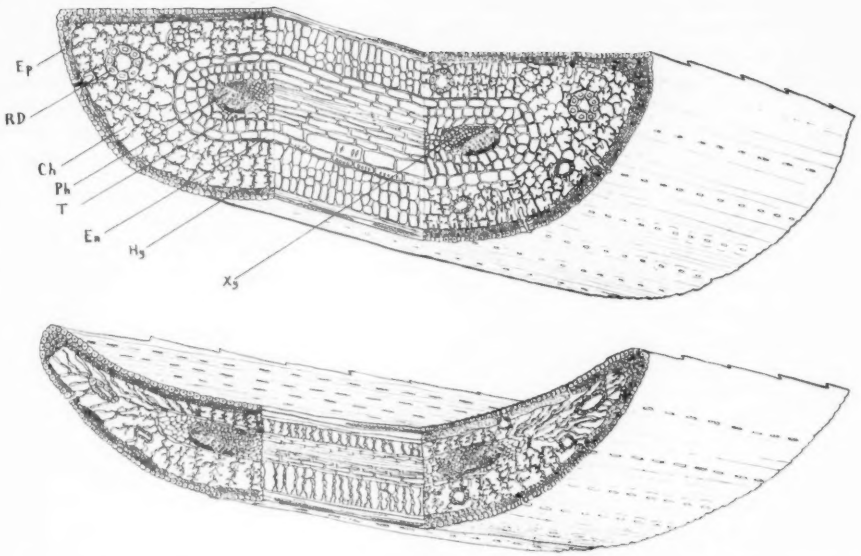

FIG. 1.—*Upper*, normal leaf of Austrian pine in cross and longisection; *Ep*, epidermis; *RD*, resin duct; *Ch*, chlorenchyma; *Ph*, phloem; *T*, transfusion tissue; *En*, endodermis; *Hy*, hypodermis; *Xy*, xylem. *Lower*, same leaf dehydrated to about 55% of original weight.

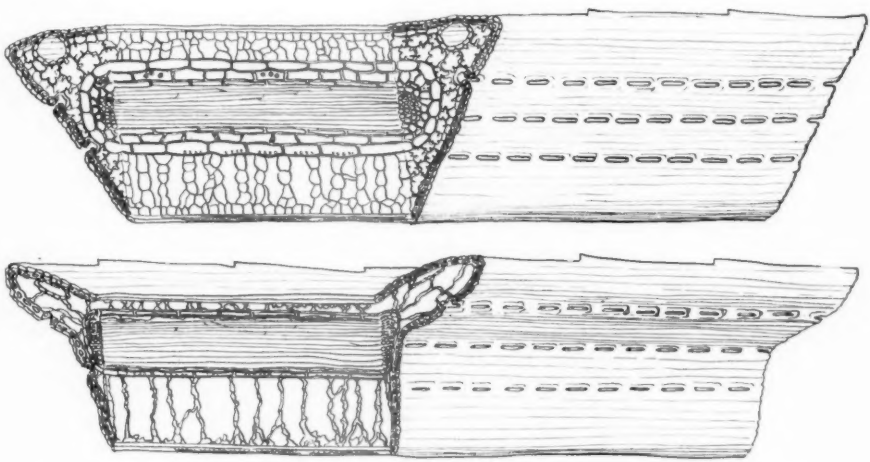

FIG. 2.—*Upper*, normal leaf of white pine in cross and longisection. *Lower*, same leaf dehydrated to about 55% of original weight.

the hypodermis depends to some extent on their position. Some chlorenchyma cells collapse lengthwise (in their longest axis), pulling the epidermis and hypodermis inward. Others stretch when they are attached to the hypodermis at the corners of the needle. In the latter case the transfusion cells shrink, and yet the corners of the needle do not bend in, necessitating a stretching of the cells attached between these two tissues. However, the speed of drying appears to cause variation in the relative rates of shrinkage of transfusion and chlorenchyma tissue (**17**). The endodermis cells also flatten out, and transfusion tracheids as well as the transfusion parenchyma collapse or are distorted. The xylem and phloem remain about the same size during desiccation.

Change in color, from grass green to light green or parrot green, during drying may be caused by the contracted condition of the chlorenchyma cells or by physical changes within the chloroplasts but not by a change in the chlorophyll structure itself. After 2 weeks of drying, the chlorenchyma cells still showed a strong primary fluorescence under the ultraviolet microscope. Needles kept in weak light (up to 500 foot-candles) remained light green and did not turn brown unless invaded by micro-organisms or subjected to certain vapors.

When air-dried leaves were placed in sterile water, they recovered their shape and color if drying had not proceeded too far; but if they had become light green and brittle, they did not ordinarily recover their shape or color after soaking in water for a week, even though they gained weight during this time. If dried, light-green leaves were cut and placed in water, they recovered their shape but not their color within 2 hours near the cut. Leaves which turned black, brown, or yellow in water were found to be infected with micro-organisms. In tissue which was thoroughly dried and then rehydrated, the chlorenchyma cells recovered their shape, but the protoplasm remained collapsed, a condition called pseudoplasmolysis (**8, 9**). If the condition persisted for several hours without recovery, the cells were considered inactive, since they showed no positive tetrazolium test unless invaded with bacteria. Figure 3 illustrates these changes.

EXPERIMENT 2.—When leaves of Austrian pine were brought into the laboratory and in 4 hours were warmed from nearly freezing to room temperature, the respiration rate showed a brief decline and then general leveling off after 1–2 days (fig. 4). As the rapidly dried needles (*D*) declined in moisture content to 85% of their original weight, a slight rise in respiration rate occurred. Subsequently, after each period of illumination this rise recurred, reaching a peak rate when the moisture content was 68%. The more slowly dried needles (*C*) did not show such a pronounced rise, and this occurred at a leaf weight of 78% of the original weight. Nineteen days after beginning the experiment no tetrazolium test was positive in the rapidly dried group, but the test was nearly fully positive in the slowly dried group. Although the *D* group showed no test macroscopically, in sectioned needles the test was still positive microscopically in the transfusion parenchyma, endodermis, phloem, and parenchyma of xylem and phloem. No positive test was found in any cells of either the *C* or the *D* group after 28 days of drying. Because of various operational difficulties, it was not possible to get reliable CO_2 measurements after this time.

EXPERIMENT 3.—When the above experiment was repeated, except that needles were kept in the dark and in

quiet air, the rise in respiration rate again occurred in the rapidly dried needles (fig. 5, treatment *A*). The peak occurred at 75% of the original weight, but with

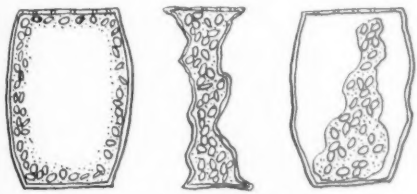

FIG. 3.—Chlorenchyma cell of Austrian pine as seen in longisection of needle: *left*, normal turgid cell; *center*, collapsed dehydrated cell; *right*, rehydrated pseudoplasmolyzed cell.

at 30 days when the tetrazolium test was negative in both groups *A* and *B*, but at 32 days no measurable amount of CO_2 was given off by either group.

EXPERIMENT 4.—When dehydration of excised leaves was carried out in quiet air of relatively high humidity (90%), there was practically no difference between Austrian and white pine in dehydration rate. But when they were dried in still air of 75.8% relative humidity over a chemical drier, or in moving air of about 80% relative humidity, white pine dried out considerably faster than Austrian pine.

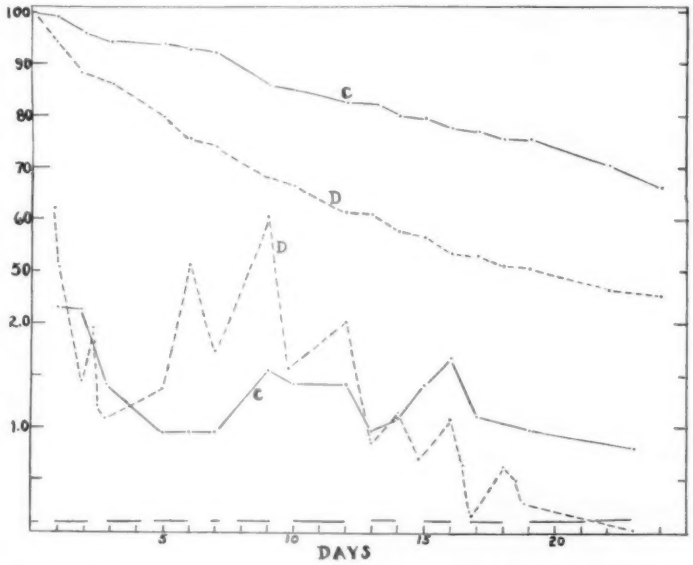

FIG. 4.—Weights of Austrian pine needles as percentage of original weight during dehydration (*above*, *C* and *D*) and respiration as μmg/CO_2 released/min/gm fresh weight for same needles (*below*, *C* and *D*). Periods of illumination shown by line at base, no illumination by blanks.

treatment *B* there was no outstanding rise in the rate. Results of the tetrazolium test carried out concurrently by sampling a few of the needles at intervals are also shown in figure 5. Unfortunately it was not possible to make a CO_2 measurement

Needles of both species, maintained at high air-moisture levels over tap water, lost or gained no appreciable weight and survived for 23 days as indicated by the tetrazolium test. Other leaves were kept in a viable condition for as long as 3

months. Slow fluctuations in moisture content of about 10% of the original weight per day did not nullify the tetrazolium test so long as the moisture content did not fall much below 75% of the original weight.

were dried 2 days in air of 93% relative humidity and rehydrated in distilled water for 1 hour or in air of 99% relative humidity for 24 hours gave a weak test.

When onion bulbs were frozen to various temperatures, some tissues were

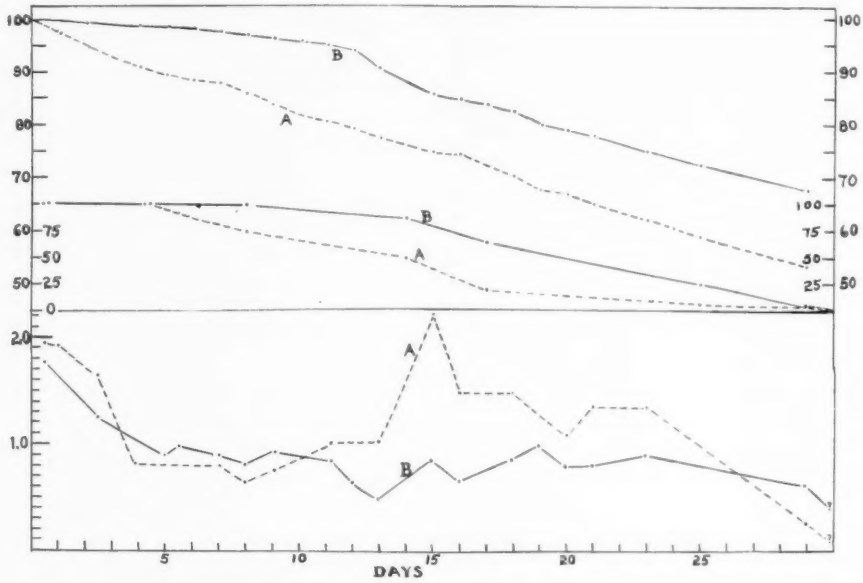

FIG. 5.—Weights of Austrian pine needles as percentage of original weight (*above*, *A* and *B*), percentage of needles sampled showing positive tetrazolium test in chlorenchyma (*middle*, *A* and *B*), and respiration as given in figure 4 (*below*, *A* and *B*).

Needles of both Austrian and white pine revealed a gradual decline in the percentage, showing a positive tetrazolium test until nearly 50% of the original weight was reached. This decline appeared first in the chlorenchyma and later in the transfusion tissue, although the chlorophyll may mask a weak positive tetrazolium test.

EXPERIMENT 5.—In young growing shoots of onions the tetrazolium test with a 1% solution gave positive results in 20 minutes. But when such tissue was dried, frozen, or boiled as described above, no test was positive. However, roots which

more easily damaged than others. Tissue damaged by cooling to −14° C. as described above gave no tetrazolium test and a very weak CO_2 release rate several hours after warming to room temperature.

The length of time onion roots were left in a 0.07% solution was roughly proportional to the inhibition of their growth. After 12 hours they turned deep red and stopped growing, but, when soaked for only 45 minutes, they continued growth although stained pink. Dr. H. ZIEGLER in the same laboratory found that the red color appeared first in the

microsomes, which in certain cells of onion roots may be seen to sink down in response to gravity, then in the chloroplasts, if present, and finally as crystals of formazan in the cytoplasm.

The author found that in leaves of conifers the color appeared slightly back from the cut ends, penetrating farthest in the central cylinder and least in the chlorenchyma. Only living cells showed the color except for the resin which also stained red in the ducts. Transfusion parenchyma cells are thus clearly differentiated from the transfusion tracheids, something that may also be done on an anatomical basis (**6**). Results of some of these observations are summarized in table 1.

TABLE 1

COLORS OF CELLS OF AUSTRIAN PINE LEAVES (AS SEEN UNDER THE MICROSCOPE) AFTER VARIOUS TREATMENTS

Treatment	Normal light, no stain			Ultraviolet light, no stain			Normal light, tetrazolium test		
	Chlorenchyma	Transfusion parenchyma	Phloem	Chlorenchyma	Transfusion parenchyma	Phloem	Chlorenchyma	Transfusion parenchyma	Phloem
Normal leaf	Green	Clear	Clear	Red	Weak red	Blue-green	Red	Red	Red
Leaf dried 4 days at 60° C, relative humidity	Green	Clear	Clear	Red	Weak red	Blue-green	Weak red	Pink	Red
Leaf dried 14 days at 60° C, relative humidity	Green	Clear	Yellow-red	Red	Weak red	Dark	Green	Clear	Yellow-red

Discussion

Tetrazolium chloride has been known as a chemical since 1894, but KUHN and JERCHEL in 1941 (**10**) were the first to report its effects in plant tissue. It is almost unique among the dehydrogenase-reduced substances in that the reduced form is colored instead of colorless. These writers found that ascorbic acid, cysteine, and glutathione (SH) do not reduce the salt if the pH value is under 9.0. Its advantages over other enzyme reactants include its nonpoisonous character to human beings and its inability to diffuse out of the cells after reduction. Its disadvantages are that it is rapidly reduced by strong light, by reducing sugars if the temperature is up to 40° C. (**3**), and by bacteria and fungi which may reduce it outside their cells (**10**).

Since the enzymes that affect it may function in vitro, it would not be surprising if otherwise dead cells could still show a positive test. However, the test was negative in cells subjected to certain heat, cold, and drying treatments. It is, of course, possible that dehydrogenase enzymes remain intact after death but that a suitable substrate is lacking in dead cells or even in the resin of resin ducts, so that the tetrazolium chloride is not reduced.

The lethal level in dehydrated pine leaves as measured by this salt does not appear to be a sharp border line but a zone of slow transition from fully active cells giving a deep carmine-red stain to weakly active ones giving a faint pink color in the same length of time. This transition may also be observed with onion tissue by the fluorochrome dye method of STRUGGER (**16**). When viewed under the ultraviolet microscope, Acredin Orange stains living protoplasm

green and varies to red in dead protoplasm. Completely erroneous results can be obtained, however, if great care is not taken to prevent damage to the thin epidermal layer of cells from the onion scale. If thicker tissues, like roots, are used, the penetration of the dye into the cells in the middle of the tissue is slow, and in an hour's time a 1:1000 solution may stain them green, thus leading the observer to think that they are alive when it is obvious that the protoplasm is completely disrupted. In conifer leaves the application of this test is especially difficult, since even the living nonchlorophyllous cells exhibit a primary fluorescence under the ultraviolet microscope as previously demonstrated by BUKATSCH (**2**) in *Eucalyptus* leaves.

The concept of slow breakdown during dehydration is also indicated by the data on CO_2 release, although an increase in the amount of CO_2 release is associated with a decrease in the tetrazolium test. This increase has been used by MONTFORT and HAHN (**12**) as a measure of the drought resistance of certain leaves. When the leaves are rehydrated, a large increase in released CO_2 is believed to indicate a relatively high sensitivity to drought.

It might be supposed that fungi could enter the leaves, if spores were not already present, and cause this rise, but no evidence of fungal invasion was found in these needles. Also, needles which were known to be infected with fungi gave off much less CO_2 than normal needles. This may be explained by the presence of large areas of dead tissue which were found to give off a barely measurable amount of CO_2. It is possible that the invasion of a fungus could cause a rise in the rate of CO_2, as FISCHER (**4**) has demonstrated with apples, but the area of the needle infected by the living fungus was relatively small in our material, and the area of dead leaf tissue and dead mycelium was always increasing so that this rise was not observed. This respiratory rise with time has also been observed in uninfected apples and is considered to be a sign of the beginning of the end of the living condition (**1**).

If the tissue is kept free of micro-organisms, the lethal level appears to be a measurable value in many mesophytes. HÖFLER *et al.* (**5**) have determined the lethal level of a number of herbaceous mesophytes. PISEK and BERGER (**14**) found that the lethal level varied beween Norway spruce (*Picea excelsa* Link) and Scotch pine (*Pinus sylvestris* L.), although they considered the relative amount of water retained by the leaves in a given time to be of great importance to continued survival during a drought. Evidently lethal levels vary among certain species of plants although no evidence of it was found here in conifers. It has long been known that drought resistance may be increased by subjecting plants to atmospheric or soil drought, but this "hardening" may consist partly of increased xeromorphism of the leaves and increased suberization of the roots (**19**). Greater water-retentive capacity is therefore to be carefully distinguished from what STOCKER called "protoplasmic" resistance. Just what happens to the protoplasm during hardening is a matter of controversy but seems to be related to the state of hydration of the protoplasm (**11**) which in turn affects the enzyme systems as they are associated with the plasma proteins and thus the respiration rate (**15**).

Summary

1. The gradual drying of excised leaves of Austrian and white pine (*Pinus nigra* Arn., var. *austriaca* Asch. et Gr. and *P.*

strobus L., respectively) is accompanied by marked shrinkage of cells of the chlorenchyma, transfusion tissue, and endodermis. Rehydration of such leaves may result in irreversible pseudoplasmolysis if dehydration is carried too far, even at the slow rate of dehydration and rehydration described. The color change from grass green to light green during desiccation appears to have a physical rather than a chemical cause, although it is usually related to loss of a positive tetrazolium test and reduction to zero of the CO_2 released.

2. The rate of CO_2 release from recently thawed, excised leaves of Austrian pine declined rapidly at first, leveled off, and then in a few days increased before decreasing to zero if they were dried rapidly. More slowly dried needles did not show this pronounced rise. Illuminating the needles between measurements caused a temporary increase in CO_2 released. More rapidly dehydrated needles lost their ability to reduce tetrazolium chloride and to produce CO_2 at lower moisture levels than the more slowly dried needles.

3. The tetrazolium test was positive in all living cells of healthy conifer leaves and also in the resin of the ducts. When the test was negative in the chlorenchyma, it was often still positive in certain cells within and including the endodermis. In onion tissue which had been damaged by various heating, cooling, and drying methods the test was weakened or failed to appear. The test itself caused a decrease in growth rate of onion roots. As a measure of viability it is much easier to use than a fluorochrome dye which probably is adsorbed on the protoplasmic particles. The tetrazolium test did not entirely follow the results of the CO_2 measurements but became weaker some time before the CO_2 release rate began to decline. As measured by these methods the lethal level appeared to be a definite range of deterioration as drying proceeded but to be somewhat variable with treatment.

The writer is much indebted to Professor BRUNO HUBER for inviting him to work in the Forstbotanisches Institut of the University of Munich and to the many members of the Institut who helped to make equipment and information available. The suggestions of Professor OTTO RENNER and the use of the Botanical Gardens are also greatly appreciated.

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FLORAL MORPHOLOGY OF THREE SPECIES OF GAULTHERIA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 638

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Introduction

Of the approximately one hundred species of *Gaultheria* which are found mainly in Asia and in Central and South America, five species occur in North America north of Mexico, namely: *G. procumbens* L., *G. ovatifolia* A. Gray, *G. shallon* Pursh, *G. humifusa* (Graham) Rydb., and *G. hispidula* (L.) Bigel. (**20, 41**). *G. hispidula* has sometimes been considered a separate genus, *Chiogenes*, and by some placed in the Vaccinioideae (**38**). *Gaultheria*, together with *Pernettya*, *Wittsteinia*, and *Diplycosia*, may either be placed in an independent tribe, the Gaultherieae, of the subfamily Arbutoideae of the family Ericaceae (**18**), or be included in the tribe Andromedeae of the same subfamily (**38**). The primary taxonomic character used to separate these two tribes is the fruit.

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

AIRY-SHAW's paper on the classification of the Asiatic species of *Gaultheria* (**1**) definitely assigns four of the North American species, whose affinities he considers to be definitely Asiatic, and tentatively assigns the fifth to positions in the five sections delimited on the basis of the Asiatic genera. The five sections with their subsections are as follows:

1. Eugaultheria
   - Procumbentes
   - Trichophyllae
   - Hispidulae
2. Brossaeopsis
   - Nummularioideae
   - Dumicolae
   - Atjehenses
3. Amblyandra
4. Gymnobotrys
5. Leucothoides

Of the five North American species, *G. procumbens* was classified in Eugaultheria, subsection Procumbentes; *G. ovatifolia* and *G. humifusa* in Amblyandra; *G. hispidula* in Eugaultheria, subsection Hispidulae; and *G. shallon* was considered as closely related to the species classified in Brossaeopsis, subsection Nummularioideae. Thus the three spe-

cies considered in this paper, *G. procumbens*, *G. ovatifolia*, and *G. shallon*, were classified in different sections of the genus.

Morphological studies have been made of members of various groups of the Ericales. PAYER (**35**) described the floral development of a few species. ARTOPOEUS (**2**) studied the dehiscence mechanism of the stamens and some aspects of seed development of several species. More comprehensive studies of staminal dehiscence were made by MATTHEWS and TAYLOR (**29**) and MATTHEWS and KNOX (**28**), and of seed development by PELTRISOT (**36**) and SAMUELSSON (**39**). HANCY (**23**) has described the vascular anatomy of a few hypogynous and epigynous species of the Ericaceae. Leaf anatomy of the Rhododendroideae was studied by BREITFELD (**3**) and of the Arbutoideae and Vaccinioideae by NIEDENZU (**31**). HAGERUP (**22**) has determined chromosome numbers in an attempt to show the relationships of the various groups of Ericales. CAMP (**6–8**) has, among other factors, evaluated chromosome numbers, population aspects, and geographic distribution in attempting to unravel the speciation in *Vaccinium*. COPELAND has studied the seed development of certain Ericales (**10**), the floral structure of *Pholisma* (**11**) (on the basis of which he believes the Lennoaceae should be removed from the Ericales), and the vegetative anatomy and various aspects of floral morphology of the Monotropoideae (**12, 13**), Rhododendroideae (**14**), and Pyroleae (**15**). For the latter three groups he has attempted a taxonomic classification using internal morphology to supplement the usual characters of gross morphology. COX (**16, 17**) has, as a result of his study of wood anatomy, proposed systems of classification for the Rhododendroideae and Arbutoideae. More recently PALSER has studied the organography, vascular anatomy, megasporogenesis, and megagametophyte development in the Andromedeae (**32, 34**) and the embryogeny and endosperm development of *Cassiope hypnoides* (**33**); and KAVALJIAN (**25**) has reported observations on various aspects of floral morphology in three species of *Clethra*.

As far as the genus *Gaultheria* itself is concerned, HANCY (**23**) described the vascular anatomy of the flower of *G. procumbens;* MATTHEWS and KNOX (**28**), the structure and dehiscence mechanism of the anther in *G. procumbens;* PELTRISOT (**36**), the development and structure of the seeds of *G. procumbens* and *G. shallon;* SAMUELSSON (**39**), the development of ovules of *G. microphylla*, *G. punctata*, *G. repens*, and *G. shallon;* and *G. shallon* is covered by MARTIN (**27**) in his paper on seed classification. Chromosome numbers of several species of *Gaultheria* have been determined by CALLAN (**5**) and NEWCOMER (**30**). There appear to be three basic chromosome numbers: 11, 12, and 13. *G. procumbens* with 24 is a diploid, while *G. shallon* with 88 (96 reported by HAGERUP [**22**]) is an octoploid. CAMP (**9**) has reported a concentration of diploid species in the southern hemisphere, with the basic number 11 occurring only in the southern three-quarters of the "Gaultheria ring" and groups with 12 and 13 in the northern part.

In many instances studies of floral morphology have been of assistance to the taxonomists. The present paper presents observations on several aspects of the floral morphology of *G. procumbens*, *G. ovatifolia*, and *G. shallon*. Although consideration of three out of the approximately one hundred species of one genus of the four is not adequate for drawing conclusions concerning classification within the genus or concerning relation-

ships between the Gaultherieae and other members of the Ericaceae, suggestions may be derived from this study which will be useful when material of other species can be obtained from South and Central America and Asia, and the study is extended to cover a more adequate representation of the genus and tribe.

Material and methods

Of the three species of *Gaultheria* (*G. procumbens*, *G. ovatifolia*, and *G. shallon*) considered in this paper, only the first is found near Chicago; the latter two are found in the Pacific Northwest. The sources of the collection used for this study were: *G. procumbens*—collections by Dr. B. F. PALSER at Beverly Shores, Indiana, Great Smoky Mountains National Park,[2] and near Berlin and Milan, New Hampshire; collections made for the taxonomy courses in the Department of Botany at the University of Chicago, probably from northern Indiana; and collections by the writer at Tremont, Indiana, at regular intervals during the summer of 1951. *G. ovatifolia*—a collection by Dr. B. F. Palser from Mount Rainier National Park[3] and the rest kindly collected and supplied by Mr. R. N. MCINTYRE, Park Naturalist of Mount Rainier National Park. *G. shallon*—collections by Dr. B. F. PALSER on Stevens Pass in the Cascade Mountains, Washington, and at Mount Rainier National Park; by Mrs. MARGARET J. HAWKES at Hood Canal; and by Dr. E. J. KRAUS at Newport, Oregon, and on the Olympic Peninsula, Washington.

Most of the collections were killed and fixed in Conant's modification of Navashin's solution, but for a small number formalin–acetic acid–alcohol was used. The former gave better results for cytological studies. All were dehydrated in an ethyl alcohol–tertiary butyl alcohol series and imbedded in a paraffin-rubber-beeswax mixture. For most tissues paraffin having a melting point of 53°–55° C. was used, but a Fisher Tissumat preparation melting at 60°–62° C. was more satisfactory for old fruits. Even in Tissumat the hard coats of the seeds within the fruits caused tearing, and thus sectioning was difficult. Some different technique needs to be found before studies can be made of the older fruits and their contained seeds. Both cross and longitudinal sections were cut, usually 10 μ in thickness but a few at 8 μ or 12 μ. Three different stains were used: KRAUS' modification of Fleming's triple, Heidenhain's iron alum–hematoxylin with Orange G, and FOSTER's (21) tannic acid-ferric chloride and safranin, the last of which has been found highly satisfactory for both anatomical and cytological study.

All drawings were made with a camera lucida, but some of the longitudinal diagrams of flower buds and an occasional megagametophyte have some details added from the adjacent sections in the series. About two hundred buds and young fruits of *G. procumbens*, sixty of *G. ovatifolia*, and forty of *G. shallon* were studied.

Observations

More material of *Gaultheria procumbens* was available; therefore as complete a description as possible is given below for this species. The descriptions of *G. ovatifolia* and *G. shallon* indicate the extent of similarity or dissimilarity to *G. procumbens* for those characteristics observed. In all three species the flower is pentacyclic, pentamerous, actinomorphic, hypogynous, and perfect.

[2] Collection made with the permission of the Park Naturalist, Mr. ARTHUR STUPKA, at Great Smoky Mountains National Park.

[3] Collection made (1948) with the permission of the Park Naturalist, Mr. RUSSELL K. GRATER, at Mount Rainier National Park.

GAULTHERIA PROCUMBENS

ORGANOGRAPHY.—The flower is pendulous, either solitary or in a small raceme of three or four flowers. The pedicel bears two opposite and ovate bracts close under the calyx (fig. 11).

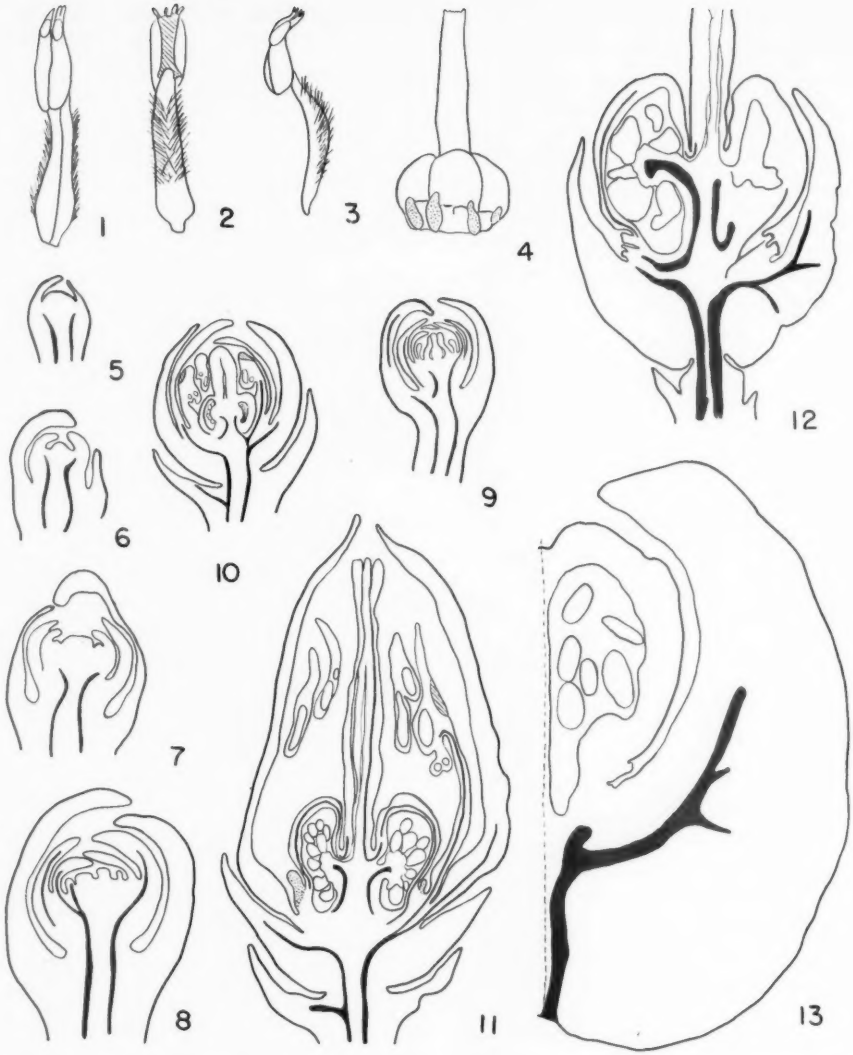
The five sepals are completely separate from one another. Their aestivation in the bud is quincuncial—that type of imbricate aestivation in which two sepals are wholly internal, two wholly external, and one is half internal and half external (fig. 20).

The ten stamens occur in two whorls of five each. The outer row, lower in origin, is opposite the petals, and the inner, upper row is opposite the sepals (figs. 21, 25–27). The filaments are either glabrous (fig. 21) or pubescent on the dorsal surfaces (fig. 2) and are considerably flattened laterally, appearing somewhat rectangular in cross section. The straight filaments meet the introrse anthers at a point about one-third of the distance from the base of the anther to the tip. Each anther contains four thecae, two on each side of the connective, and is located above the top of the ovary (fig. 24). In older anthers the two thecae on each side of the connective become confluent, so that each shedding anther has only two pollen sacs (fig. 34*a*). Dehiscence is accomplished by the formation of terminal pores. The mechanism of dehiscence was described by MATTHEWS and KNOX (**28**), and the present material corroborates their report. The microspores do not separate after meiosis, so that the shedding pollen is in the form of tetrahedral tetrads.

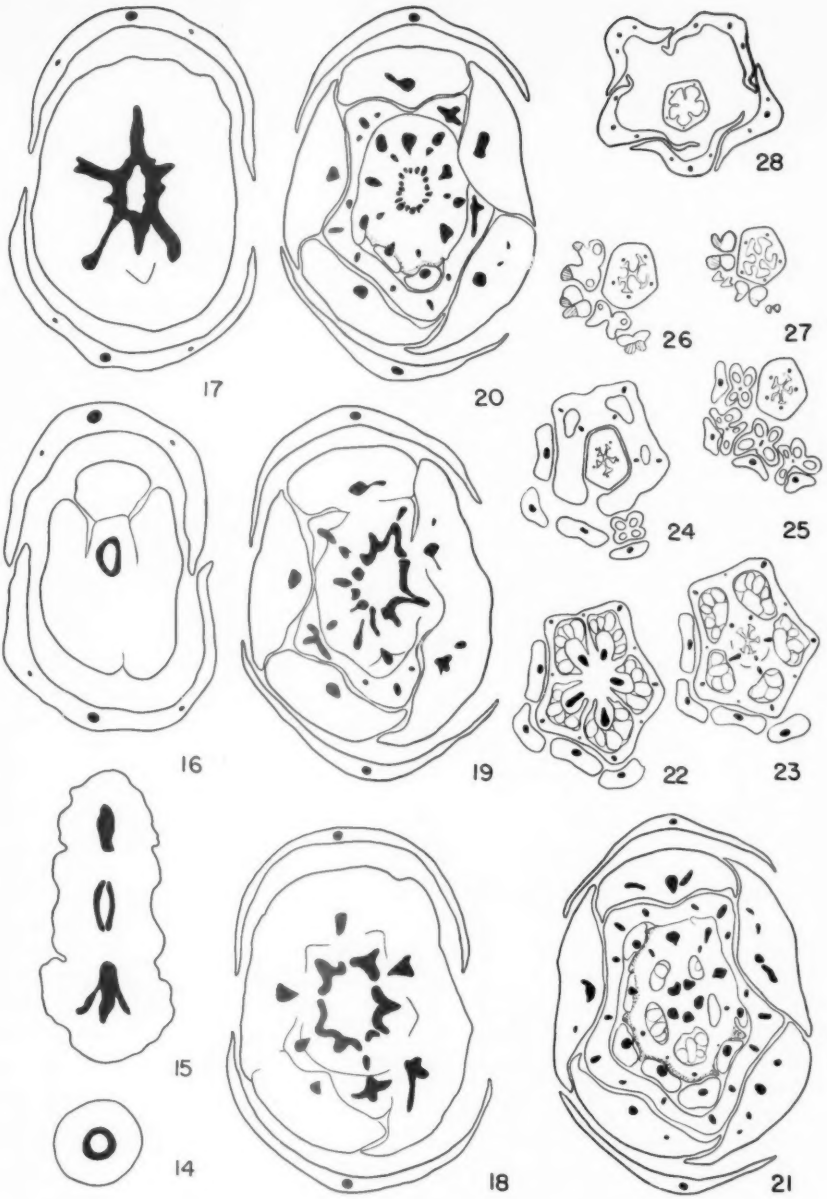
Each stamen bears a pair of appendages, or awns (figs. 1–3, 26, 27, 33), each consisting primarily of a pouch of highly granular, eventually disintegrating cells (fig. 34*a*). The disintegrating region is continuous from the lateral edges of the filament just about the region of its attachment to the anther through a protruding area of the connective to a continuation of this connective tissue above the level of the thecae where the appendage bifurcates. This description corroborates that of MATTHEWS and KNOX (**28**).

The ovary is globular and has a depression at the top from which the style emerges. The style is straight, with a slight expansion just above the ovary, and terminates in an unexpanded stigma just below the level of the corolla opening (fig. 11). The ovary is five-carpellate, with the five locules occurring opposite the petals (fig. 21). The five pendant, axile placentae arise from the upper portion of a central column with each placenta occupying about one-third of the locule area of a single carpel and bearing five to ten ovules on the outer surface between it and the dorsal wall (fig. 22). The ovules are closer to anatropous (fig. 39*a*) than to any other type, but because there is a bend in the chalazal portion of the megagametophyte, they cannot be considered as strictly anatropous. SAMUELSSON (**39**) has reported that anatropous ovules occur in *G. shallon*, *G. microphylla*, *Pernettya mucronata*, and *P. pumila*.

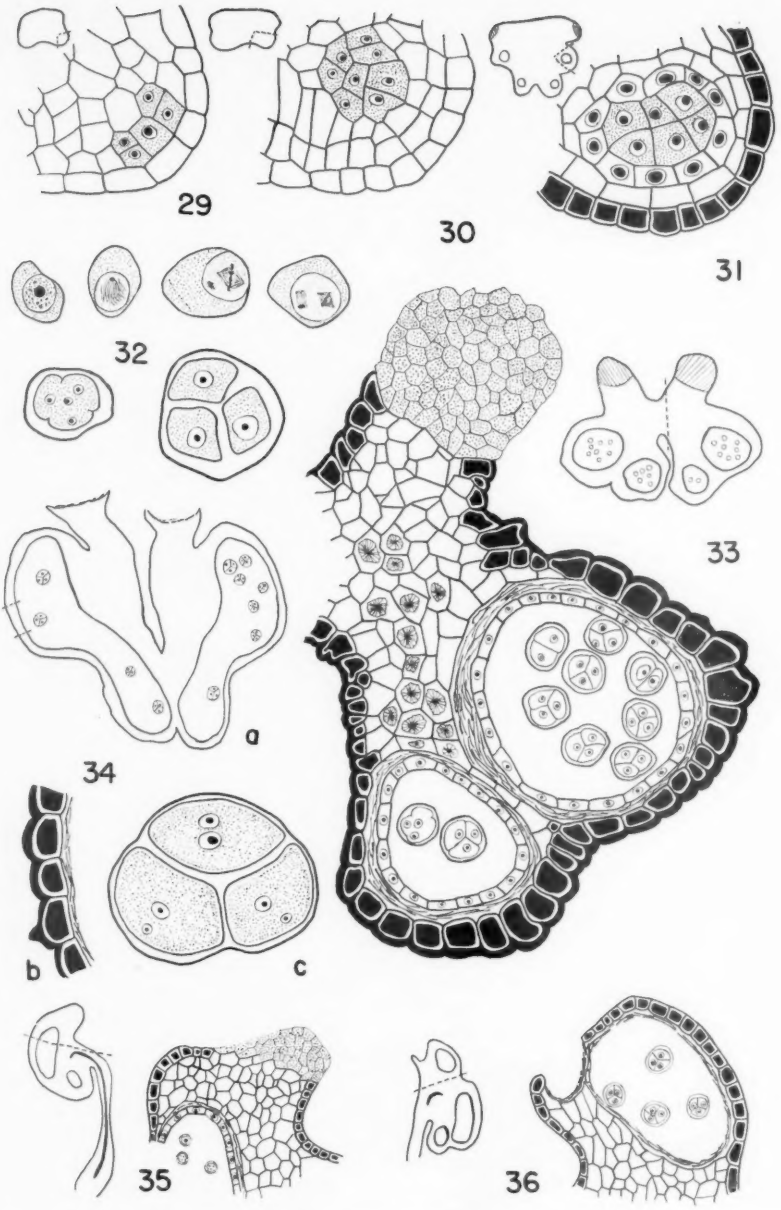
A split develops in the tissue of the central column, above the level of the placentae, and extends radially in the plane of the placentae, thus becoming continuous with the locules. It is continuous into and through the style, forming a five-angled stylar canal (figs. 23–28). The angles of the canal correspond to and are continuous with the locules, and the tissues between the angles of the canal correspond to the septa between the carpels. The canal is almost completely closed in the young buds (fig. 10) but opens in the older flowers (fig. 11). A mass of loose papillate cells is formed on the surface of the stigma.



FIGS. 1–13.—*Gaultheria procumbens*. Stippled areas indicate nectariferous tissue, and single-hatched areas indicate disintegration tissue of awns. Figs. 1–3, adaxial, abaxial, and lateral view of stamen, ×13. Fig. 4, pistil and nectary lobes, ×13. Figs. 5–13, floral development from initiation to fruit: figs. 5–8, ×57; figs. 9–13, ×27. Fig. 5, flower primordium with bracts. Fig. 6, young flower with sepal primordia. Fig. 7, young flower with sepals, petals, and stamen primordia. Fig. 8, young flower showing initiation of carpels. Fig. 9, young flower with initiation of bend in stamen and placenta in ovary. Fig. 10, longisection of bud. Fig. 11, longisection of flower. Fig. 12, longisection of young fruit. Fig. 13, longisection of fruit not yet mature.



FIGS. 14–28.—*Gaultheria procumbens*. Diagrams of serial cross sections of bud from pedicel to corolla opening showing details of vascular anatomy and certain features of organography. Only ovary and four of ten stamens are shown in figs. 22–27. ×41.



FIGS. 29–36.—Fig. 29–34, *Gaultheria procumbens*, development of anther and microsporogenesis. All diagrams, ×123; details except 33 and 34*b*, ×1279; and 33 and 34*b*, ×535. Fig. 29, cross section of anther showing archesporial, primary parietal, and primary sporogenous cells. Figs. 30, 31, stages in development of sporogenous tissue and wall layers. Fig. 32, microspore mother cell, meiosis, and tetrad of microspores. Fig. 33, cross section of an almost mature anther; note granular character of cells in awn. Fig. 34, mature anther: *a*, diagram with disintegrated awn and fused thecae; *b*, detail of wall layers; *c*, mature pollen grain. Figs. 35, 36, *Gaultheria ovatifolia*, longisections of anther. Diagrams, ×59, and details, ×319. Fig. 35, young anther showing small awn. Fig. 36, mature anther showing disintegrated area of awn.

A nectariferous enlargement of the external wall to the base of the ovary appears in all except the very young buds. There are usually ten small free lobes of nectariferous tissue which project upward from the ovary wall, alternating with the filaments (fig. 4).

The fruit is berry-like, usually bright-red or occasionally white, with a large fleshy calyx inclosing a loculicidal capsule. Immediately after fertilization the five sepals become fleshy and fuse with one another from the base upward; however, no fusion occurs between the calyx and the ovary wall (figs. 12, 13).

FLORAL DEVELOPMENT.—Flowers are initiated on the new growth of the current year which, in the Chicago area, comes above ground during June. The buds develop fairly rapidly, opening in mid-July, and the fruit is mature in September, though it may remain attached to the plant until the following spring. Each individual flower originates as a rounded protuberance which early becomes inclosed within a pair of bracts (fig. 5). The primordium enlarges considerably before the sepals are initiated. The latter are followed successively in an inward and somewhat upward direction by the primordia of petals, stamens, and finally carpels (figs. 6–9).

The stamens are initiated as small protuberances (fig. 7) and then elongate. Before the archesporial cells are recognizable, the terminal portion of the stamens starts to bend (fig. 9). By the time sporogenous tissue is distinct in the microsporangia, the bend is almost complete, and a few darkly stained cells have appeared in the tips of the two projecting arms of the connective. These cells are differentiated in the connective and extend vertically up to the distal end of the anther from a point a little above the junction of the filament and the anther. This is the earliest indication of the awns. Cross sections of mature anthers show that cells of the connective tissue adjacent to the granular cells stain darkly and are thick-walled. These thick-walled areas can be traced along the length of the anther beyond the fertile lobes into the two terminal protruding areas, each of which bifurcates beyond the level of the thecae.

Five separate carpel primordia are initiated (fig. 8); they gradually unite into a sac, of which the upper edge elongates to form the style, and the lower portion enlarges to form the ovary (fig. 9). On the inner surface of the sac five vertical swellings appear along the lines of fusion of the carpel primordia. These septal primordia grow toward the center of the sac and fuse, thus forming five compartments in the sac cavity. The small cavity in each compartment enlarges to form a locule, and the placenta arises from the central column. Ovules are initiated on the placenta only after sporogenous tissues have been differentiated in the anthers.

Microsporogenesis precedes megasporogenesis. The tetrads of microspores have already been formed in the anther before meiosis of the megaspore mother cell occurs.

VASCULAR ANATOMY.—In the pedicel the vascular tissues form a continuous cylinder (fig. 14). This is interrupted by gaps resulting from the departure of traces to the bracts (fig. 15). For each bract a single trace leaves the central cylinder and divides immediately into three bundles. The median bundle extends to the tip of the bract, but the lateral ones are very short. The bract gaps close, and a complete vascular ring enters the receptacle (fig. 16) where it broadens and gives rise to the traces to the floral organs. None of the latter traces shows

reflexion in this species, and all have a definite upward trend.

Vascular traces are given off in essentially acropetal order to the sepals, petals, petalad stamens, sepalad stamens, and to the dorsal, septal (lateral), and ventral regions of the carpels. Only four whorls of bundles depart from the central cylinder, however, leaving a small amount of vascular tissue in the center which constitutes the ventral carpellary supply. The number of whorls departing is lower than the number of organs supplied because the traces to petals and petalad stamens, and to sepalad stamens and the septal regions of the carpels, arise as common bundles.

The vascular supply to each sepal is a single trace (fig. 17) leaving a single gap. The bundle remains undivided or occasionally branches into three in the sepal itself, the two small laterals extending only a short distance.

The vascular supply to each petal is also a single trace (fig. 18), the five petal traces alternating with the sepal traces. Each bundle divides into three in the base of the petal, and the three bundles of each petal traverse the corolla (figs. 18–21).

The single trace to each petalad stamen has a common origin with that of the petal and separates tangentially from the common bundle near its origin (fig. 11). Each stamen bundle remains undivided through the filament and into the connective where, just before termination of the bundle, it has a sharp downward bend (figs. 11, 25).

Traces to the sepalad stamens, a single bundle to each stamen, arise in the plane of the sepal traces after sepal gaps have closed (fig. 19). The course of each bundle is similar to that described for the petalad stamen.

The dorsal carpellary bundles are small and arise in the plane of the common petal-petalad stamen traces before the closure of the petal gap. They arise independently from one side of the gap (fig. 19), though the side from which they arise may vary. The bundles pass undivided through the carpel wall, turn under the depression at the top of the ovary, and enter the style, where each bundle is located at the end of an angle of the stylar canal (figs. 22–28). They continue undivided through the length of the style and fade out a little distance below the stigma.

Five smaller septal carpellary bundles alternate with the dorsal carpel bundles (figs. 22, 23). They have a common origin with the sepalad stamen traces, from which they diverge near the separation of filaments from the receptacle (figs. 20, 21). The bundles pass upward in the septal region of the wall to the top of the ovary, where they fade out without reaching the base of the depression or entering the style.

The vascular tissue remaining in the center constitutes the ventral carpellary supply (fig. 20). It turns inward and then upward where it forms an essentially continuous ring of vascular tissue. Higher, the cylinder separates to five distinct bundles corresponding to the five locules (fig. 21). Each bundle is the total ventral supply of a single carpel and turns into the placenta where it branches to form the vascular supply to the individual ovules.

The nectariferous tissue receives no vascular supply.

This description corroborates that of HANCY (**23**) with respect to sepal, petal, stamen, and septal carpel supply. She did not describe the dorsal or ventral carpel bundles, however, though they are shown in her diagrams; gaps formed in the re-

ceptacular cylinder were neither shown nor mentioned.

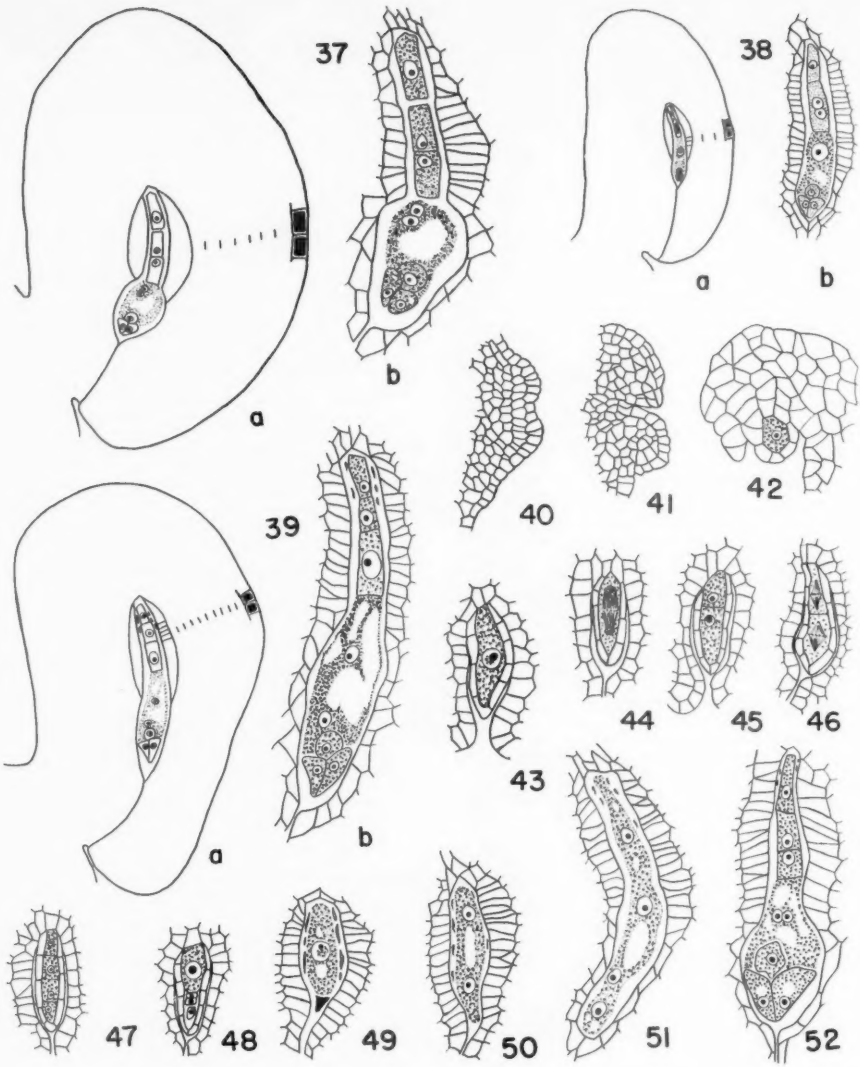
MICROSPOROGENESIS.—A cross section of the young anther shows four lobes, each of which represents a microsporangium. In each microsporangial area a few hypodermal cells enlarge to form the archesporial tissue which is continuous vertically for a considerable distance. Each archesporial cell divides periclinally into an outer primary parietal cell and an inner primary sporogenous cell (fig. 29). The primary parietal cells divide once periclinally; of the resulting cells, the outer layer underlying the epidermis forms a wall layer which disintegrates late in development, and the inner layer forms the tapetum in which the cells become large and isodiametric and have densely granular cytoplasm and conspicuous nuclei, one per cell (figs. 30, 31). These cells remain intact until the maturation of the spores, though they become flattened at the time of meiosis (fig. 33). They eventually separate from one another and disintegrate (fig. 34*b*).

Further divisions of the primary sporogenous cells give rise to sporogenous tissue and eventually to the microspore mother cells (figs. 30, 31, 32). There are about five to twenty of the latter visible in a cross section of a single theca. The microspore mother cells undergo meiosis (fig. 32), and the resulting microspores show a tetrahedral arrangement. The spores enlarge considerably in size and remain attached to one another, so that the shedding pollen is a tetrad (fig. 34*c*). Within each spore the nucleus divides to form a generative and a tube nucleus. Formation of distinct cells was not observed, but special stains were not used to try to determine this point.

Tannin or tannin-like substances begin to be deposited in the epidermis of both anther and filament at about the time the tapetum is differentiating (fig. 31). Druses have been found in many connective cells (fig. 33), but they seem to disappear just before dehiscence. The occurrence of druses was not reported by MATTHEWS and KNOX (**28**).

MEGASPOROGENESIS AND DEVELOPMENT OF MEGAGAMETOPHYTE.—In the development of the ovule the nucellus appears first as a small protuberance on the surface of the placenta (fig. 40). On this the single integument arises very early as a small outgrowth near the base (fig. 41). By the time the single hypodermal archesporial cell can be distinguished from the surrounding nucellar cells by its large size and denser cytoplasm, the integument is obvious, particularly on the outer curve of the bend of the ovule (fig. 42). The archesporial cell enlarges rapidly, mostly by elongation, giving rise directly to the megaspore mother cell which is thus surrounded by a single layer of nucellar cells (fig. 43). During the enlargement of the archesporial cell to form the megaspore mother cell the integument becomes four or five cell layers in thickness, elongates rapidly to completely surround the nucellus, and forms the micropyle (fig. 43). During development of the megagametophyte the ovule increases considerably in size and completes its bending to the anatropous condition, and the integument increases in thickness so that it has twelve layers of cells by the time the megagametophyte is mature (fig. 39*a*). The cells of the outer layer of the integument, except for the few around the micropyle, contain a considerable deposit of tannin or tannin-like substances.

The elongated megaspore mother cell undergoes the first meiotic division, forming a dyad (figs. 44, 45), and the second meiotic division results in a linear tetrad (figs. 46, 47). The three micropylar



FIGS. 37–52.—Fig. 37, *Gaultheria shallon:* *a*, diagram of longisection of mature ovule, ×308; *b*, detail of mature megagametophyte of *a*, ×517. Fig. 38, *Gaultheria ovatifolia:* *a*, diagram of longisection of mature ovule, ×308; *b*, detail of mature megagametophyte of *a*, ×517. Figs. 39–52, *Gaultheria procumbens*. Fig. 39*a*, diagram of longisection of mature ovule, ×308; *b*, detail of mature megagametophyte of *a*, ×517. Figs. 40–52, stages in development of ovule and megagametophyte, ×517. Fig. 40, initiation of ovule. Fig. 41, bending of ovule and initiation of integument. Fig. 42, archesporial cell. Fig. 43, megaspore mother cell. Fig. 44, first meiotic division. Fig. 45, dyad. Fig. 46, second meiotic division. Fig. 47, tetrad. Fig. 48, degeneration of micropylar megaspores. Fig. 49, functional megaspore, nucellus degenerated, epithelial-like layer differentiated. Fig. 50, two-nucleate megagametophyte. Fig. 51, four-nucleate megagametophyte. Fig. 52, mature megagametophyte.

megaspores degenerate, leaving the chalazal one as the functional megaspore (fig. 48).

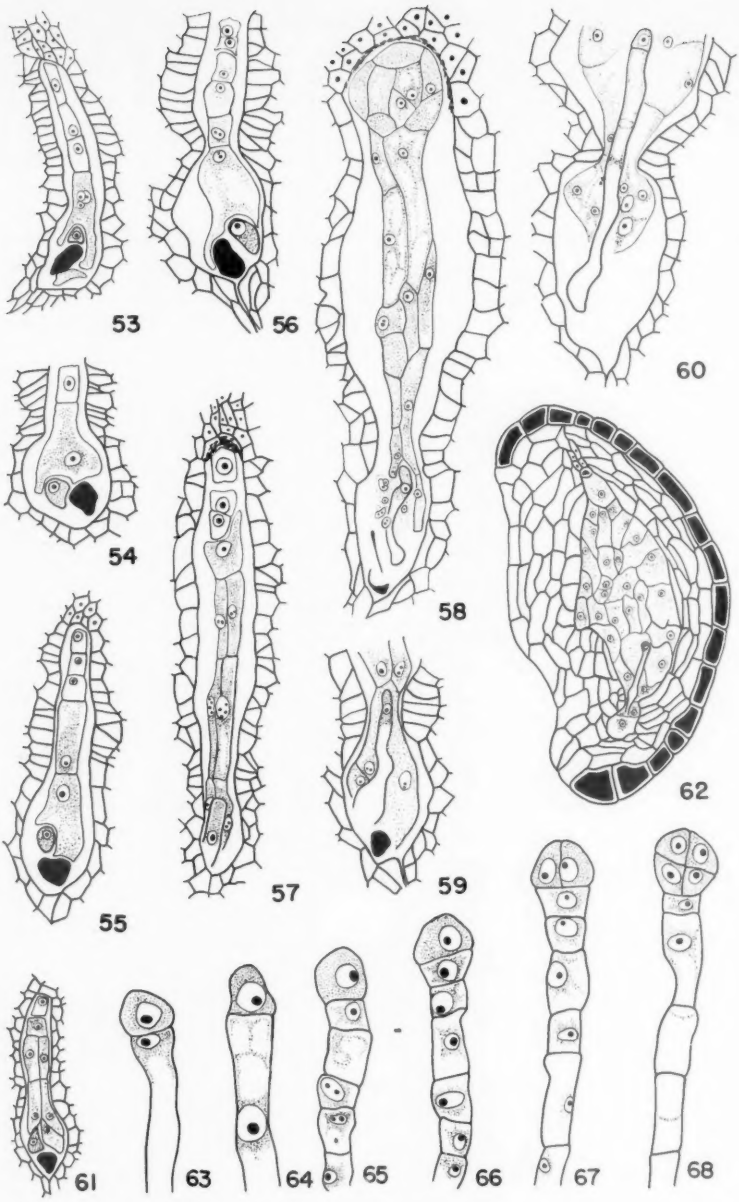
After the functional megaspore has enlarged considerably and is about ready to divide, the nucellus begins to disintegrate. Simultaneously that part of the inner epidermis of the integument which surrounds the nucellus differentiates as an epithelial-like layer characterized by columnar, compact, densely cytoplasmic cells (fig. 49).

During enlargement of the functional megaspore a vacuole appears at either end of the cell with the nucleus remaining in the center (fig. 49). The nucleus divides into two which come to lie at either end of a rather large central vacuole. The resulting two-nucleate gametophyte (fig. 50) projects beyond the original limits of the nucellus toward the micropyle. The two nuclei divide to form a four-nucleate gametophyte (fig. 51) in which the two pairs of nuclei are separated by the large central vacuole. The gametophyte projects so far micropylarly that only the two chalazal nuclei are inclosed by the epithelial-like layer. A slight bending of the chalazal end occurs at this time. A third mitotic division produces eight nuclei. All three divisions occur in relatively close succession. Wall formation, occurring after the last division, leads to the formation of seven cells (figs. 39, 52). Thus, the development is of the Normal (*Polygonum*) Type (**26**).

The micropylar end of the elongated mature megagametophyte is much more expanded than the bent chalazal end and appears to have crushed the adjacent cells of the integument. The egg apparatus consists of an oval-shaped egg and two synergids which appear triangular in longitudinal section. The polar nuclei migrate toward each other and occupy a position at the anterior end of the narrow part of the gametophyte which is surrounded by the epithelial-like layer of the integument. The three antipodal cells are arranged linearly and are so large that they occupy one-third to one-half of the total length of the megagametophyte. SAMUELSSON (**39**) has reported similarly enlarged antipodals in *G. shallon*, *G. microphylla*, and *Pernettya mucronata*.

EMBRYOGENY AND ENDOSPERM DEVELOPMENT.—The pollen tube enters the ovule through the micropyle and in penetrating the megagametophyte occasionally destroys one but more frequently both synergids. The fusion of one sperm and the egg, and of the second sperm and the two polar nuclei, occurs rapidly, the former somewhat more rapidly than the latter (fig. 53). After fertilization the embryo sac contains a zygote and the remains of the pollen tube and synergids at the micropylar end, a primary endosperm nucleus near the center, and the three, unchanged antipodals at the chalazal end (figs. 53, 54). Remains of the pollen tube and synergids may persist after distingegration of the antipodals and during formation of the prominent micropylar and chalazal haustoria (fig. 58).

During the development of zygote and endosperm, expansion both anterior and posterior to, but not in the region of, the micropylar cells of the epithelial layer of the integument results in a constriction near the micropylar end of the embryo sac (fig. 54). The region anterior to the constriction becomes the micropylar haustorium. During early development the zygote is located anterior to the constriction, and the primary endosperm nucleus posterior to it (fig. 54). The inner layer of the integument loses its epithelial appearance as a result of enlargement of the endosperm, and the integumentary cells surrounding the chalazal



FIGS. 53–68.—Figs. 53–60, *Gaultheria procumbens*, stages in endosperm development and embryogeny, ×413. Figs. 53, fertilization, zygote fused, primary endosperm fusing, antipodals showing. Fig. 54, zygote and primary endosperm nucleus with beginning of micropylar haustorium, one antipodal showing. Fig. 55, zygote and two-celled endosperm, antipodals showing. Fig. 56, zygote and four-celled endosperm, two antipodals showing. Fig. 57, zygote beginning to elongate, and seven-celled endosperm, antipodals showing. Fig. 58, elongation of zygote, multicellular endosperm with micropylar and chalazal haustoria well developed. Fig. 59, further elongation of zygote. Fig. 60, first division of zygote. Figs. 61–68, *Gaultheria ovatifolia*, endosperm development and embryogeny. Fig. 61, zygote and six-celled endosperm, one antipodal showing, ×413. Fig. 62, elongate zygote, development of seed showing multicellular endosperm with micropylar and chalazal haustoria, ×246. Figs. 63–68, stages in development of embryo, ×986.

end of the embryo sac become highly cytoplasmic and their nuclei conspicuous (fig. 55).

The initial stages of endosperm development precede those of the zygote. Immediately after fertilization both ovule and embryo sac elongate. The first division of the primary endosperm nucleus is oriented parallel to the long axis of the embryo sac and is followed by wall formation, thus dividing the embryo sac into two chambers of approximately equal length (fig. 55). A second division, somewhat earlier in the micropylar cell than in the chalazal, results in the formation of a linear row of four cells (fig. 56). The micropylar cell of the four-celled endosperm, with its cross wall located just posterior to the constriction, has dense cytoplasm, is large in size, irregular in form, and surrounds the zygote. The chalazal cell is the smallest of the four and extends back into the region occupied by the antipodals.

First the micropylar cell and then the two central cells of the four-celled endosperm divide longitudinally, the chalazal cell remaining undivided, though extending further posteriorly (fig. 57). Details of subsequent divisions could not be determined from the available material, but observation of older fruits suggests that divisions take place rapidly and in various planes anterior to the constriction, resulting in the formation of numerous endosperm nuclei with indistinct walls in the micropylar haustorium. Eventually the chalazal cell also undergoes divisions in various planes, forming the chalazal haustorium, which becomes a region of numerous, densely cytoplasmic cells separated from the endosperm proper by a constriction and surrounded by a layer of crushed tissue made up of antipodals and integumentary cells (fig. 58). The main body of the endosperm is derived, by many divisions in all planes, from the central cells. SAMUELSSON (**39**) reported a linear row of four endosperm cells and two endosperm haustoria characteristic of *G. shallon*, *G. punctata*, *G. repens*, and *Pernettya mucronata*.

Initially the zygote is an essentially globular cell located anterior to the micropylar constriction. At about the four-celled stage of the endosperm it starts to elongate toward the chalaza with the greater part of the cytoplasm and the nucleus remaining in the tip of the cell (fig. 59). Elongation continues until the tip penetrates through the constriction into the main body of the endosperm to about the level of the second cell of the original endosperm quartet. Here the first division of the zygote, a transverse one, cuts off a small terminal cell from a much elongated basal cell (fig. 60). Technical difficulties prevented observation of the subsequent stages in development.

GAULTHERIA OVATIFOLIA

ORGANOGRAPHY.—The flower is solitary in the axil of a leaf. Its pedicel bears two or three pairs of alternately opposite bracts, the uppermost just a little below the calyx. The pedicel and outer surfaces of the sepals are covered with multicellular hairs (figs. 76, 77); other floral organs are glabrous. The five sepals are undiverged for a very short distance from the base, and the aestivation is valvate. The five petals, each with a saccate base, alternate with the sepals. Though free from one another at the base, they are joined above to form a prominently five-lobed, campanulate corolla. The free limbs show valvate aestivation. As in *G. procumbens*, the ten stamens occur in two cycles. Each filament has an enlarged basal portion and meets the small introrse anther near its mid-point. In young buds each anther bears two small awns (fig. 35) which dis-

integrate during maturation, so that in the flower the anther appears awnless, although careful observation will show a little residue (fig. 36). The structure of the ovary and of the style is the same as in *G. procumbens*. Each locule contains fifteen to twenty-five small ovules. The style terminates in the stigma at a level about two-thirds of the total length of the young flower. The scarlet fruit is similar in structure to that of *G. procumbens*.

VASCULAR ANATOMY.—The vascular supply of pedicel and bracts is similar to that of *G. procumbens*. The sepal supply arises as a single broad trace, leaving a single gap, and immediately separates into three (figs. 69, 70). All three traces, especially the lateral ones, curve downward in their outward course before turning up in a normal position (fig. 69). Further branching results in five to seven bundles in each sepal. The petal, petalad stamen, sepalad stamen, and septal and ventral carpellary traces are similar to those in *G. procumbens* except that (*a*) the petal bundles do not divide into three each until well up in the corolla and that (*b*) the septal bundles, instead of ending at the top of the ovary, turn under the depression and enter the style, where they fade out a little lower than the dorsal carpellary strands (fig. 72). The dorsal carpellary traces arise independently, as in *G. procumbens*, but each has a double origin—part of the strand arising from each side of the petal gap before the closure of the latter (fig. 71).

MICROSPOROGENESIS.—In the youngest buds observed the sporogenous tissue had already differentiated. The development from sporogenous tissue to mature pollen grain is similar to that of *G. procumbens* except that the tapetum disintegrates earlier (at the time of meiosis). No druses have been found in the connective of the anther.

MEGASPOROGENESIS AND DEVELOPMENT OF THE MEGAGAMETOPHYTE.—Complete development has not been followed. The megaspore mother cell and the two-nucleate megagametophyte are similar to those of *G. procumbens*. In the mature megagametophyte the polar nuclei fuse before fertilization, and no wall separates the first and second antipodal nuclei (fig. 38*b*). The single integument consists of five layers of cells (fig. 38*a*).

EMBRYOGENY AND ENDOSPERM DEVELOPMENT.—The youngest endosperm seen was six-celled—four tiers of cells with the central two divided longitudinally (fig. 61). In a later stage (fig. 62) a micropylar haustorium, consisting of two cells, and a chalazal one, of a single cell, are differentiated. The main body of the endosperm is made up of numerous cells.

The first division of the zygote is transverse, as in *G. procumbens*, cutting off a small terminal cell from a much elongated basal cell (figs. 63, 64). The plane of the second division, and of a few subsequent ones, is transverse, but whether it occurs in the terminal or basal cell is not known (figs. 65, 66). When the embryonic filament is seven cells long, the distal cell—the embryo proper—divides longitudinally (fig. 67); the remaining cells constitute the suspensor. The two-celled embryo divides to four and then eight cells (fig. 68), but the suspensor shows no further divisions. Subsequent developmental stages were not available. MARTIN (27) described the mature embryo of *Gaultheria* as small, straight, with the two cotyledons inconspicuously developed.

GAULTHERIA SHALLON

ORGANOGRAPHY.—The flowers are in scaly-bracted, raceme-like panicles which arise from scaly buds. The pedicel bears a pair of scaly bracts just below the

calyx. The pedicel, outer surfaces of sepals and petals, and sometimes the dorsal surface of the filaments are heavily pubescent. The hairs are multicellular, most of them with enlarged glandular tips (fig. 74), except on the filaments, where they are unicellular (fig. 75). The five sepals are undiverged for a short distance from the base and are valvate in aestivation. Petals and stamens are similar to those of *G. procumbens* except that the awns protrude farther above the thecae. Ovary and style are also similar and ovule number just slightly higher (eight to sixteen). The fruit, also a loculicidal capsule covered by a fleshy calyx, varies from purple to black.

VASCULAR ANATOMY.—The vascular supply of pedicel and bracts is similar to that in *G. procumbens*. As in *G. ovatifolia*, the sepal supply arises as a single trace, immediately divides into three strands which enter the base of the sepal and may there redivide into five, sometimes seven or more, bundles. Also as in *G. ovatifolia*, all sepal traces, especially the lateral ones, are reflexed. The dorsal carpel bundle in the species has a common origin with that of the petal and petalad stamen (fig. 73). The common bundle is reflexed; the petal trace diverges first from the common strand near the top of the curve, and then the strands to the petalad stamen and the dorsal region of the carpel diverge from each other near the separation of the filament from the receptacle. Each petal trace divides into three strands after entering the base of the petal, and some bundles divide again, with the result that the total number of petal bundles at higher levels is irregular. The vascular supply to the sepalad stamens and septal and ventral regions of the carpels is as in *G. procumbens*.

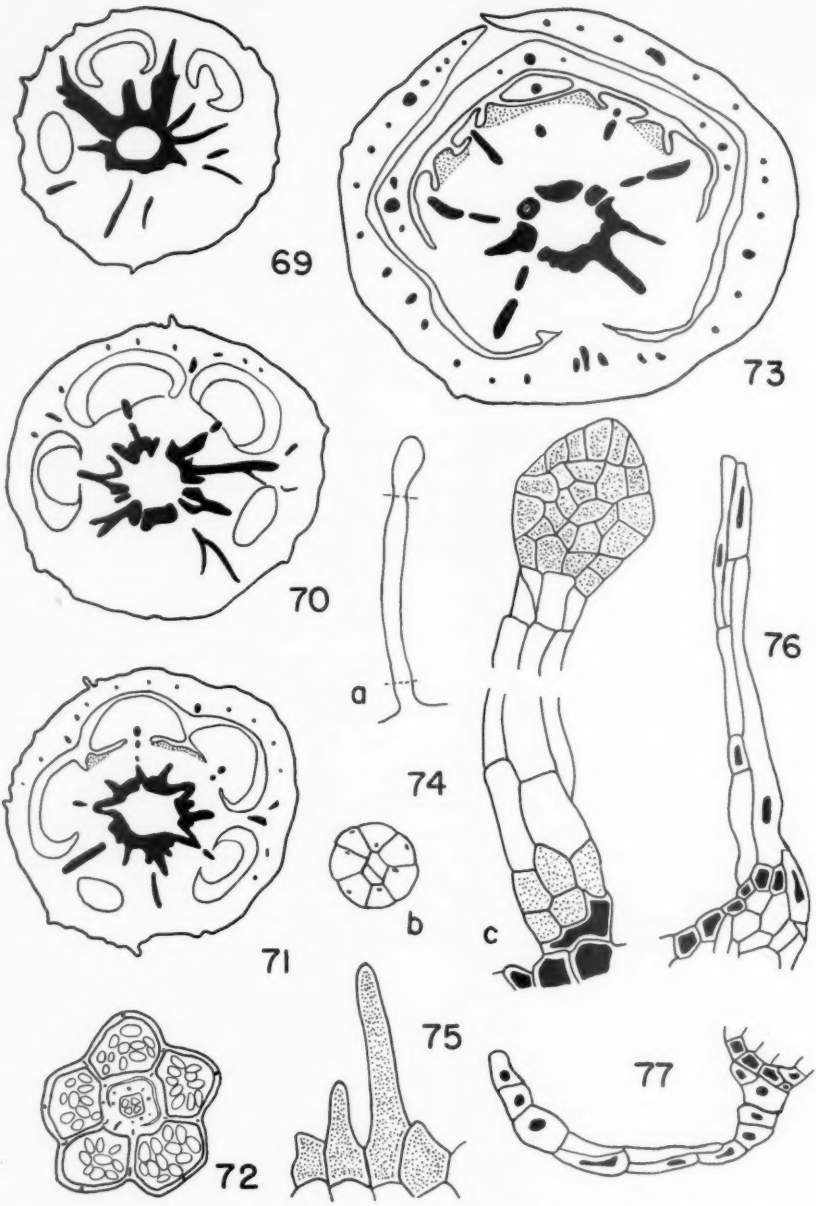
MICROSPOROGENESIS. — Microsporogenesis in *G. shallon* is similar to that in *G. procumbens*, except that the tapetum disintegrates earlier, as in *G. ovatifolia*. In some anthers the wall layers may not be completely crushed at the time of dehiscence.

MEGASPOROGENESIS AND DEVELOPMENT OF MEGAGAMETOPHYTE.—Development of and within the ovules corresponds closely to that in *G. procumbens*. The epithelial-like layer of the integument differentiates earlier (at the tetrad stage), however, and the micropylar portion of the mature megagametophyte is more expanded with the two polar nuclei situated slightly anterior to the constriction and the three antipodals occupying the narrow, more bent chalazal area (fig. 37*b*). When the megagametophyte is mature, the single integument consists of nine layers of cells (fig. 37*a*).

Discussion

As indicated in the introduction, *Gaultheria* and related genera have been classified either in an independent tribe or included in the Andromedeae. Whether they should be considered as an independent tribe depends on the whole of their similarities or dissimilarities to the Andromedeae and on the relationship of these features to criteria that are accepted as valid in separating groups of tribal rank.

Taxonomically, the Gaultherieae are differentiated from the Andromedeae primarily by the character of their fruits. In the Gaultherieae the loculicidal capsule is partially to almost completely enveloped by a persistent calyx which becomes fleshy and gives the fruit the appearance of a berry. In contrast, the loculicidal capsule of the Andromedeae has no such fleshy covering. The fruit of the Arbuteae, a third tribe of the same subfamily, is a drupe containing four or five nutlets, with the calyx usually small and disklike



FIGS. 69–77.—Figs. 69–72, 76, 77, *Gaultheria ovatifolia*. Figs. 73–75, *Gaultheria shallon*. Figs. 69–72, cross section of bud, ×32. Figs. 69, 70, section through receptacle to show origin of sepal traces. Fig. 71, section at higher level to show origin of dorsal carpel traces. Fig. 72, section at top of ovary to show septal carpel traces entering style. Fig. 73, cross section of bud, showing origin of dorsal carpel traces, ×59. Fig. 74, multicellular, glandular hair: *a*, diagram of longisection, ×142; *b*, cross section of stalk, ×620; *c*, detail of base and tip of *a*, ×620. Fig. 75, longisection of unicellular hairs, ×620. Figs. 76, 77, longisection of multicellular hair, ×370.

on the base. Fruits of three other subfamilies of the Ericaceae differ from those of the Arbutoideae. The Rhododendroideae and Ericoideae are characterized by septicidal capsules; the Vaccinioideae, by a berry. The fruits of most of the Monotropoideae and Pyroleae, which groups COPELAND (**12, 13, 15**) has suggested have been derived from the Andromedeae, also have loculicidal capsules. COX (**17**) has substantiated the maintenance of the four genera, *Gaultheria, Pernettya, Wittsteinia*, and *Diplycosia*, as an independent tribe of the Arbutoideae on the basis of wood anatomy.

To determine whether details of the vascular anatomy of the flower and of general embryology can contribute either to the status of the Gaultherieae as a tribe or add additional characters for the support of AIRY-SHAW's taxonomic treatment of *Gaultheria*, a comparison must be made among the three species studied and of these three species with other members of the Ericales, particularly of the Andromedeae.

As has been suggested by EAMES and MACDANIELS (**19**) and by PURI (**37**), a consideration of the course of the vascular supply to the various floral organs can be of value in comparative studies of particular groups of plants. It is generally considered that three traces, with three associated gaps, form the primitive vascular supply to a sepal (**15, 19**). This condition is found in a few members of the Ericaceae: *Chimaphila* (**15**), *Bejaria* (**14**), *Leucothoë racemosa*, and *L. recurva* (**32**). Two patterns of reduction of the three traces have been suggested by PALSER (**32**). One of these is seen in the sepal supply in *G. ovatifolia* and *G. shallon:* "The origins of the three traces to one sepal have come closer and closer together, until the three depart as a common bundle, leaving a single gap in the cylinder. This bundle separates into its three component strands while still in the receptacle." In these two species of *Gaultheria* each sepal originates as a broad strand which immediately separates into its three component parts. This would suggest that the reduction has not progressed very far. *G. procumbens*, with its single trace which either remains undivided or divides only well out in the sepal, has carried the reduction much further and, in this respect, can be considered more advanced than the other two species.

The type of sepal supply in *G. ovatifolia* and *G. shallon* is rather similar to that in *Leucothoë axillaris* and *L. catesbaei* of the Andromedeae (**32**) and in *Elliottia, Menziesia, Rhododendron*, and *Ledum* of the Rhododendroideae (**14**); the type in *G. procumbens* is similar to that in *Andromeda* and *Cassiope* of the Andromedeae (**32**), in several species of *Vaccinium* and *Gaylussacia* of the Vaccinioideae (**23**), and in a few of the Rhododendroideae and Monotropoideae (**12–14**).

Since a reduction from three traces to one per sepal has been seen within such groups of the Ericaceae as the Rhododendroideae and Andromedeae, as is indicated above, it will be interesting to see if study of other species of *Gaultheria* will reveal some with three independent traces, or if all of them will show reduction along the one line indicated by the three species studied in this paper.

A single trace supplies the petal in all ericad species that have been reported, with the exception of *Chimaphila*, where COPELAND (**15**) reported one to three traces. In some species the single trace divides into three while still in the receptacle, but in many, as in *Gaultheria*, branching occurs only in the base of the petal or at an even higher level.

In all species that have been described a stamen is supplied by a single bundle which bends downward in the connective before its termination. In a few, though not in *Gaultheria*, there is also an upward extension of the bundle (**14, 25**).

The origin of dorsal carpel traces is variable, the only common feature being that they arise in the plane of the petals. This is seen in the three species of *Gaultheria* reported on herein: in one the dorsal carpel trace has a common origin with the petal-petalad stamen bundle; and in the other two, while arising independently before the closure of the petal gap, it originates in one from one side of the gap, and in the other from both sides.

Variation is as great within the Andromedeae (**32**), where it may vary within the species or occasionally even within a single flower. For example, in *Leucothoë axillaris* and *Ampelothamnus phyllireifolius* it arises sometimes from one side of the gap, sometimes from both sides. Species in which the origin is consistent (as in *Gaultheria*) are not lacking, however. For example, in *Pieris japonica* the bundle always arises from one side of the gap; in *Andromeda glaucophylla* and *A. polifolia* it always arises from both sides of it; and in *Cassiope hypnoides* and *Lyonia mariana* it has a common origin with the petal-petalad stamen trace.

Septal carpel bundles have been described in all reports of the vascular anatomy of the flower in the Ericaceae. The bundles frequently do not branch and usually terminate without entering the style. The latter is not the case, however, in *G. cvatifolia* where the septal carpel bundles turn under the depression at the top of the ovary, enter the style, and fade out at a little distance below the stigma. This has been reported only by COPELAND (**13, 15**) in the Pyroleae and *Sarcodes* and by KAVALJIAN (**25**) in *Clethra arborea*.

The ventral carpel bundles in *Gaultheria* have "fused" in the plane of the locules of the carpel, as in most of the Andromedeae and in the Rhododendroideae.

The degree of nondivergence among the various strands to the floral organs is quite varied in the Ericales. In some of the Andromedeae (**32**), for example, all floral whorls are supplied independently, while at the other extreme the petal, petalad stamen, and dorsal carpel traces and the sepal and sepalad stamen traces have a common origin. In many the septal carpel traces may arise in common with independent sepalad stamen traces. A completely independent origin of all traces is considered indicative of primitiveness, and the greater the degree of nondivergence, the greater the degree of advancement. *Gaultheria* thus shows considerable advancement, as petal and petalad stamen traces, and sepalad stamen and septal carpel traces have a common origin in two of the species studied, while in the third the dorsal carpel traces arise in common with the petal-petalad stamen supply (as great a degree of nondivergence as found in any of the Andromedeae).

MAHESHWARI (**26**) has listed a number of embryological features as of major taxonomic value. These include, among others, certain characters of the anther, ovule structure and development of the megagametophyte within it, certain endosperm characteristics, etc. As he has suggested, these are used to delimit the larger plant groups and, on the whole, are so similar within an order that they do not provide traits of taxonomic importance for distinguishing among taxa of lower rank. Minor variations do occasionally occur, however. Certain features

in particular may be useful at the lower level in the Ericales: (*a*) time of disintegration of the nucellus; (*b*) extent of differentiation of an integumentary epithelial-like layer; (*c*) degree and time of enlargement of the megagametophyte; (*d*) time of fusion of the polar nuclei; (*e*) degree of development of the antipodals; and (*f*) degree and time of development of the endosperm haustoria.

The prevalence of appendaged anthers in the Ericales is reflected in the old ordinal epithet, Bicornes. They are found, however, only in the family Ericaceae (in the broad sense), not in the other families of the order: Clethraceae, Diapensiaceae, Epacridaceae, and Empetraceae. Exceptions within the Ericaceae are the entire subfamily Rhododendroideae and most of the Monotropoideae. The appendages occurring in the Andromedeae have been called by PALSER (**32**) either "spur" or "awn," depending on their position: "spur" for those appendages, two per stamen, of somewhat varying length and character which arise from the filament, the connective, or the anther itself close to the region of filament-anther junction and extend downward; and "awn" for the continuation of sterile tissue beyond the top of the thecae of the anther. Based on this distinction, the present species of *Gaultheria* are considered to bear awns. In structure they are essentially similar in the three species, but in *G. ovatifolia* they are very small and disintegrate before the flowers are mature—hence AIRY-SHAW's (**1**) classification of *G. ovatifolia* as awnless. Their structure in *Gaultheria* differs from that in most of the Andromedeae, but they have certain features in common with a few others, for example, *Oxydendrum* and *Leucothoë recurva* (**32**). The presence of considerably reduced appendages which are observable only in young buds, as in *G. ovatifolia*, is found in *Lyonia ligustrina* and *L. mariana*, though the appendages of the latter are somewhat different in type.

The introrse condition of the anthers in many of the Ericaceae has been shown by MATTHEWS and KNOX (**28**) to be acquired during development with the result that the apparent apex of the mature anther is morphologically the base, and the apparent base is the morphological apex. *Gaultheria* shows an early inversion of the anthers. This is also true in most members of the Andromedeae, but in *Cassiope* the inversion is not so complete as in other members and occurs relatively late in development. In *Clethra* (**2, 25**), the Pyroleae (**2, 15**), and Arbuteae (**2**) the inversion occurs at the time of anthesis, so that during the entire development of the stamen the anther is extrorse and becomes introrse only in the open flower.

An endothecium, characteristic of the anthers of so many angiosperms, is rare in the Ericales. It has been reported only by COPELAND (**14**) in *Bejaria*, *Rhodothamnus*, and *Phyllodoce* of the Rhododendroideae and by KAVALJIAN (**25**) in *Clethra*.

Variations in microsporogenesis are few. In most species that have been studied (**14, 15, 25**) the tapetum is at least binucleate, not uninucleate as here reported for *Gaultheria*. In *G. ovatifolia* and *G. shallon* the tapetum disintegrates earlier than in *G. procumbens*.

Pollen grains in the majority of the Ericaceae, including *Gaultheria*, are shed as tetrads of two-nucleate cells. Exceptions do occur: in *Chimaphila* the tetrads are easily disrupted into single grains (**15**), and single grains are characteristic of *Ramischia* (*Pyrola*) *secunda*, *Erica stricta*, and the Monotropoideae (**39**). In other families of the order tetrad pollen grains are found only in the Empetra-

ceae, not in the Clethraceae (**25, 39**), Diapensiaceae, or Epacridaceae (**39**).

General ovary characteristics are similar throughout the Ericaceae. Ovules are unitegmic and anatropous to campylotropous. Megagametophyte development is of the Normal (*Polygonum*) Type (**26**). Of the species that have been studied, only *Cassiope mertensiana* (**34**) is reported as showing important variation from the typical *Polygonum* pattern. The nucellus disintegrates early, and the inner layer of the integument becomes more or less differentiated as an epithelial-like layer surrounding at least the chalazal portion of the megagametophyte. The disintegration and differentiation occur earlier in *G. shallon* (tetrad stage) than in *G. procumbens* (functional megaspore stage). The polar nuclei may fuse (as in *G. ovatifolia* and several of the Andromedeae) or may not fuse (as in *G. procumbens* and *G. shallon* and others of the Andromedeae) prior to fertilization. According to PELTRISOT (**36**) and SCHNARF (**40**), the antipodals are small and degenerate rather early in most cases. This is not true of *Gaultheria* in which the antipodals are prominent and persist until the endosperm has divided to form six or more cells.

In the Andromedeae (**34**) the character of the antipodals varies from none remaining in the mature gametophyte, as in *Cassiope*, to poorly developed and early degenerating ones in *Andromeda*, *Oxydendrum*, etc., to three well-developed ones in *Chamaedaphne*, *Leucothoë axillaris*, etc., culminating in the multiplied antipodals found in *Leucothoë racemosa* and *L. recurva*. Only in the latter two does antipodal tissue occupy as much of the total gametophyte area as in *Gaultheria*, though in total characteristics the gametophyte in *Chamaedaphne* shows closer resemblance to that in *Gaultheria*. The stages during which elongation and expansion of the micropylar portion of the megagametophyte in *Gaultheria* occur (four-nucleated and mature) correspond more closely to those in *Leucothoë catesbaei* and *Lyonia mariana* than to those in *Chamaedaphne* (two-nucleated).

With the exception of PELTRISOT (**36**) and BROUGH (**4**), authors (**2, 10, 13–15, 39, 40, 42**) have described endosperm development in the Ericales as cellular, the first two divisions resulting in a linear tier of four cells. SAMUELSSON (**39**) has stated that the endosperm in the mature seed is derived entirely from the middle two of the four cells and that the two terminal ones give rise to haustoria. That this is true for *G. procumbens* and *G. ovatifolia* has been shown in the present paper. There appears to be variation in the plane of the divisions immediately following the four-celled stage, however. The first division may be transverse in the chalazal cell as in *Cassiope hypnoides* (**33**), longitudinal in the central cells as in *G. ovatifolia*, etc. This has not been followed closely in enough species to allow adequate comparison.

All members of the Ericales, except the Diapensiaceae (**39**), are characterized by the development, to a greater or lesser extent, of micropylar and chalazal endosperm haustoria. The micropylar haustorium is frequently larger and contains more cells than the chalazal. In *G. procumbens* both haustoria are well-developed, multicellular structures; in *G. ovatifolia* both are rather poorly developed; in *Cassiope hypnoides* (**33**) the micropylar one is well developed though with few cells, while the chalazal one is poorly developed; and in the Pyroleae (**15**) both are extremely small. The time of appearance of the haustoria is variable. The region of the micropylar haus-

torium may be apparent even before fertilization and the chalazal only later, as seen in *Gaultheria* and less clearly in *Cassiope hypnoides* (**33**); or neither may appear until after the endosperm has undergone considerable development, as in *Epigaea repens* (**42**).

Little has been done on the embryogeny of the Ericales. This is reflected in JOHANSEN's (**24**) listing of only a few members. He classified the pattern of embryo development in *Pyrola rotundifolia* as a *Myriophyllum* Variation of the Caryophyllad Type, in *Styphelia longifolia* as a *Vaccaria* Variation of the same type, in *Monotropa hypopitys* as probably of the Onagrad Type, in *Sarcodes sanguinea* as probably as the Caryophyllad Type, and in *Diapensia lapponica* as probably of the Solanad Type. Insufficient detail concerning the sequence of divisions in the very young embryo of both *G. procumbens* and *G. ovatifolia* precludes the classification of these species under any of JOHANSEN's types. The first division of the zygote in both species is undoubtedly transverse. Later divisions were observed only in *G. ovatifolia*. As the basal cell does not seem to take part in formation of the embryo, the development would be of the Solanad Type, if the first division of the terminal cell is transverse; of the Onagrad Type, if longitudinal. Proembryos are linear, but whether the cells are derived by transverse division of the terminal or of the basal cell is not known. Eventually a longitudinal division does occur in the distal cell of the filament. Thus whether the suspensor is derived from the basal or from the terminal cell of the two-celled proembryo becomes critical before development can be classified in one or the other type.

As this discussion indicates, study of these three species of *Gaultheria* has not added greatly to the solution of taxonomic problems concerning the genus. *G. procumbens* appears distinct from the other two on the basis of sepal supply; the reduction in the development of awns and the increased vascular supply to the style resulting from a continuation of the septal carpel strands serves to segregate *G. ovatifolia;* and the increased nondivergence of traces and probably the high degree of polyploidy which has been reported delimit *G. shallon*. There is no assurance, however, that these characters will occur in other species of *Gaultheria* classified by AIRY-SHAW (**1**) in the same sections and subsections of the genus as these three.

No new criteria for distinguishing the Gaultherieae from the Andromedeae have been added. Any feature characteristic of all three species of *Gaultheria* is found developed to some extent in one or more species of the Andromedeae, a tribe which, as it is now constituted, is highly variable. The Gaultherieae thus remain separated from the Andromedeae primarily on the basis of fruit structure and wood anatomy.

It is hoped that further critical study of many more members of the Gaultherieae and of some additional species and features of the Andromedeae may make a later comparison more fruitful.

Summary

1. Various aspects of the floral morphology of *Gaultheria procumbens* L., *G. ovatifolia* A. Gray, and *G. shallon* Pursh are presented.

2. These three species of *Gaultheria* agree with most members of the Ericaceae in being pentacyclic (the outer cycle of stamens opposite the petals), pentamerous, actinomorphic, sympetalous, hypogynous, and perfect. The fruit is a loculicidal capsule covered by a fleshy calyx, a character used taxonomically to

place the genus, with three others, in a separate tribe of the Arbutoideae.

3. In the receptacle the vascular cylinder gives rise to a whorl of five sepal bundles, a whorl of five petal-petalad stamen bundles, a whorl of five sepalad stamen-septal carpel bundles, and a whorl of dorsal carpel bundles (arising independently in *G. procumbens* and *G. ovatifolia* but in common with the petal-petalad stamen supply in *G. shallon*). The remaining vascular bundles form the ventral supply of the carpels. The single sepal bundle is undivided in the receptacle of *G. procumbens* but divides into three in the other two species. Stylar bundles are continuations of only the dorsal bundles except in *G. ovatifolia* where the septal bundles also continue into the style.

4. The introrse anthers are awned in all three species, though the awns are small and disappear early in *G. ovatifolia*. The general anther structure, microsporogenesis, and mature pollen are similar to those in most members of the Ericales.

5. The ovules in the three species are unitegmic, tenuinucellate, and essentially anatropous. Megagametophyte development is of the Normal (*Polygonum*) Type. Antipodals are large and persistent.

6. The endosperm is cellular and forms both micropylar and chalazal haustoria, which are large in *G. procumbens*, small in *G. ovatifolia*. The first division of the zygote is transverse. A suspensor is formed which places the young embryo within the main body of the endosperm.

7. A comparison of morphological characteristics between the Gaultherieae, as exemplified by these three species, and members of the Andromedeae show no new significant differences.

8. Morphological differences among the three species show a slight corroboration of AIRY-SHAW's taxonomic separation of them into three different sections.

The writer is greatly indebted to Professor B. F. PALSER of the Department of Botany at the University of Chicago, under whose guidance this research was undertaken and whose help in drafting the present manuscript was especially valuable. Sincere thanks are extended to all who collected the materials which helped to make this paper possible.

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FLORAL MORPHOLOGY OF POPULUS DELTOIDES AND P. TREMULOIDES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 639

M. NAGARAJ

Introduction

In the vicinity of the University of Chicago where the cottonwood, *Populus deltoides* Marsh., is a common street tree, catkins on staminate trees expand and mature 4–6 weeks earlier than those on pistillate trees. Frequently wind and rain remove essentially all staminate catkins from the trees 2 or 3 weeks prior to expansion of the pistillate catkins. At the time seeds are shed in June, however, there appears to have been little failure in seed-set as judged by the amount of cotton in the air. Assuming that pollination, if it occurs, is by *P. deltoides* pollen rather than by that of some other species, there are four possible ways of accounting for the seed-set: (*a*) few seeds are actually set, and the abundant cotton is not accompanied by fertile seed; (*b*) pollination is by pollen either caught in bud scales or on bark or blown about in the air for some time after the staminate catkins are shed; (*c*) pollination is by pollen blown in from other areas where the staminate catkins mature at the time the stigmas of the pistillate catkins are receptive; and (*d*) facultative apomixis. Considerable cotton must be examined for the presence or absence of well-developed seeds to test the first hypothesis. The second possibility has been suggested by HEIZMANN and SHULL (**14**), who observed that the pollen was very light and thought that it was probably blown about by the wind for a considerable time after the staminate catkins fell so that the pistillate flowers were always pollinated. If so, pollen must remain viable for the time elapsing between shedding and pollination. To substantiate the third hypothesis, it would be necessary to find staminate trees mature at the time of receptivity of the pistillate flowers and within a radius feasible for the carrying of rather large amounts of pollen by the wind, as well as to determine the amount and nature of the pollen occurring in the air at this critical period. To test the last possibility—facultative apomixis—it is necessary to determine what happens primarily during megasporogenesis, megagametophyte development, and early embryogeny, while information on microsporogenesis and pollen viability might be useful.

The present paper results from an attempt to determine at least partially the nature of seed-set in cottonwood. Certain gross observations and the results of a study of pollen germination are reported. More extensively all available features of floral morphology are presented with a comparison of similar features of the trembling aspen, *Populus tremuloides* Michx., which gives no indication of the time lag in catkin maturation observed in *P. deltoides*.

Populus, with about thirty species of shrubs and trees, belongs to the family Salicaceae, in which the only other genus is *Salix*. The plants are dioecious with

the naked flowers borne in slack and pendulous catkins or spikes. The flowers are solitary, each accompanied by a bract borne near the pedicel base. Stamens and pistils arise from a cup-shaped disk which develops at the end of the pedicel.

Studies of embryology in the Salicaceae have been reviewed by SCHNARF (**28**) and, of other phases of floral morphology, by HJELMQVIST (**15**). Details of floral anatomy have been reported for several species of both *Salix* and *Populus* by FISHER (**8**), of microsporogenesis for four species of *Salix* by CHAMBERLAIN (**4**), of megasporogenesis and megagametophyte development for several species of *Salix* by CHAMBERLAIN (**4**), HÅKANSSON (**12**), MAHESHWARI and ROY (**24**), and for two species of *Populus* by GRAF (**10**), and of embryogeny for some species of *Salix* by CHAMBERLAIN (**4**) and SOUÈGES (**31**). Except for the study of floral anatomy by FISHER, no detailed reports of other phases of floral morphology have been found for *P. deltoides* and *P. tremuloides*.

Material and methods

Material for study was collected from trees within a radius of about 40 miles of the University of Chicago. Collections of staminate flowers of both cottonwood and trembling aspen, from several localities, began on March 25, 1951, and were made at intervals of 3–4 days until early May. Sample collections of pistillate flowers of cottonwood were made during the spring of 1949 within a radius of a few blocks of the University, but collections at regular intervals began on April 10, 1950, and were made every 3 or 4 days until the end of May. In 1951 collections began on April 15 and were made every 3 or 4 days until June. Most of the latter material was obtained in Elgin, Illinois, and at the Morton Arboretum, Lisle, Illinois, with permission of the director. Collections of pistillate flowers of trembling aspen began on April 3, 1951, and were made every 6 or 7 days until the end of May, mostly near Tremont, Indiana. A few collections of both staminate and pistillate buds of both species were made in summer, autumn, and winter. These furnished a nearly complete developmental series from floral initiation through early embryogeny.

Material was fixed in Conant's modification of Navashin's solution. Whole catkins or individual buds were imbedded in paraffin, the 1949 and the 1951 collections after dehydrating in an ethyl alcohol–tertiary butyl alcohol series, the 1950 collections in an ethyl alcohol–xylene series. Both methods were satisfactory. Cross, longitudinal, and oblique sections cut at 10–24 μ were stained in Heidenhain's iron-alum-haemotoxylin and counterstained with gold orange. Some sections were stained with tannic acid—ferric chloride and safranin (**9**) which proved especially good for studies of vascular anatomy. All drawings except figures 1 and 16 were made with a camera lucida.

Cottonwood pollen for germination experiments was collected from anthers which were just dehiscing. For germinating the pollen the sugar-agar technique of EIGSTI (**6**), SMITH (**30**), and MYRBERG (**26**) was used throughout rather than the older hanging-drop technique (**3, 29**) which has been shown to be unsatisfactory in certain cases.

For the majority of germination tests three drops of a 10–15% sucrose and 2% agar solution (recommended by TOKUGAWA [**33**] and MYRBERG [**26**]) were brushed onto each slide with a camel's-hair brush. After the agar had partially solidified, pollen from anthers to be test-

ed was dusted across the agar smear. Light inoculations gave the most consistent results. Inoculated slides were placed on sheets of moistened filter paper in large Petri dishes which then were placed in constant-temperature chambers within 3 minutes of inoculation time. Temperatures at the level of the slides were 11°, 20°, and 30° ± 1° C.

Growth of pollen tubes was stopped after 16–20 hours by removing the slides from the germination chambers and placing them for 5–7 minutes in a drying oven at 70° C.; this satisfactorily dried the agar without cracking it. The slides were then fixed in an aceto-carmine mounting medium (**34**). This method prevents both disturbance of the tubes from the original growth pattern and loss of ungerminated grains, both of which often occur with a dip-staining method.

For storage purposes pollen was collected primarily from flowers in which the anthers had only recently opened. Storage followed the method of HOLMAN and BRUBAKER (**17**), namely, in small glass cylinders (so-called "shell vials") which were closed with corks. These corks had been bored before use, and the hole loosely plugged with a thin layer of cotton. Pollen was kept under three different conditions: (*a*) at 11° C. in complete darkness; (*b*) at 20° C. in complete darkness; (*c*) at outdoor temperatures, with dark and light varying characteristically with the season. All three groups of pollen were stored under air-dry conditions. Germination tests using the stored pollen were made at intervals of 3 or 4 days for about 1 month after collection.

Observations

Although staminate and pistillate catkins usually develop and mature at different times in the University area as indicated in the introduction, in the spring of 1951, both in Chicago and in surrounding areas, sites were found where both matured at the same time. Near Elgin, about 40 miles from the University of Chicago, this was a common feature, especially along the Fox River.

In 1951 cotton was collected from pistillate trees close to staminate trees which had mature catkins at the time the stigmas were receptive. This cotton showed seeds containing well-developed embryos attached to the piece of placenta. There was no reason to suspect that the embryos were not normal.

The description of cottonwood and trembling aspen that follows is based both on fresh material and on sections.

ORGANOGRAPHY.—The number of flowers in the pistillate catkins ranges from about forty to sixty in cottonwood and from about ninety to one hundred or more in trembling aspen. Each pistillate flower consists of a pedicel upon which is borne the bract. The pedicel, short at the time of ovule initiation, later elongates and becomes very conspicuous in the mature flowers. At the summit of the pedicel its peripheral tissues are prolonged to form the obliquely lengthened, cup-shaped disk characteristic of the flowers of *Populus* (fig. 1). The disk surrounds the basal half or two-thirds of the ovary. The pistil is practically sessile and consists of the ovary, a short style, and a deeply lobed stigma.

The ovary is two- to four-carpellate in cottonwood and always two-carpellate in trembling aspen. The placentation is parietal. Each placenta bears about eight to fifteen anatropous ovules in cottonwood, six to seven in trembling aspen.

In cottonwood the stigma has two to four very large segments. In trembling aspen it has two segments, much smaller than in cottonwood, with a smaller

middle lobe between each of these larger segments. In both species the stigma is shed early.

In both cottonwood and trembling aspen the number of flowers in a staminate catkin ranges from about eight to one hundred or more. Although similar in origin and structure, the staminate and pistillate flowers differ somewhat in shape. The disk of the staminate flower (fig. 16) is much more oblique than that of the pistillate flower.

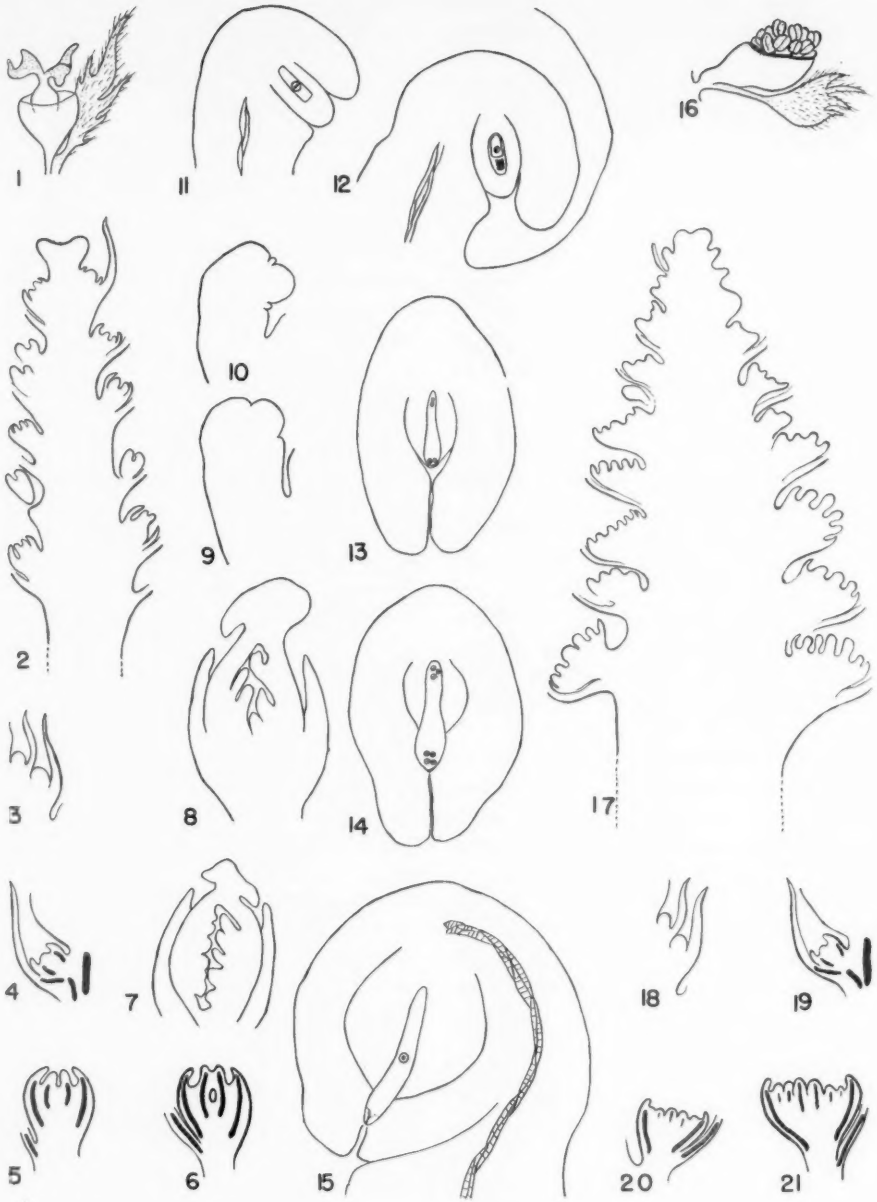
The stamens spring from the central part of the disk (fig. 21). Each flower contains forty to sixty or more stamens in cottonwood, and only five to fourteen in trembling aspen. The anther has four thecae, two on each side of the connective, each pair becoming united into a single sac a short time before the pollen is shed. The filament elongates slightly just before anthesis. Dehiscence of the anther is longitudinal. The pollen is shed as single grains.

FLORAL DEVELOPMENT.—In both cottonwood and trembling aspen the pistillate catkins arise toward the end of summer, and the staminate catkins toward the beginning of summer. Both types remain as buds during the winter and complete their development early the following spring, with anthesis occurring in midspring. The differentiation of individual flowers in the catkin is essentially acropetal and spiral (figs. 2, 17). Each individual flower appears first as a blunt protuberance on the axis of the catkin. A bract is found near the base of the pedicel (figs. 3, 18).

In pistillate buds of both species collected in August the carpels were slight protuberances but showed no trace of ovules (figs. 4, 5, 6). In October collections the ovular nucellus was conspicuous (figs. 7, 8), but the integuments had not begun to form. The latter do not develop until the following spring.

In cottonwood, during March, or sometimes as late as April, the inner integument arises as an outgrowth from the nucellar epidermis (fig. 9) and is immediately followed by the outer integument (fig. 10). The inner integument is weakly developed and consists of only one or two layers of cells. The outer integument is only two cells thick at the micropylar end in very young ovules but grows rapidly and becomes six or seven cells thick by the time the megaspore mother cell is fully differentiated (fig. 11). The inner integument fuses with the outer, sometimes prior to the megaspore-mother-cell stage, sometimes later, but at and after the two-nucleate megagametophyte stage the ovule always appears to be unitegmic (figs. 11–15). The micropyle, which is straight, closes during the two-nucleate stage and is therefore formed only by the outer integument. In older ovules the integument becomes much thicker at the micropylar end by the formation of additional layers of cells. In trembling aspen, on the other hand, there is a single integument which arises as an outgrowth from the epidermis at about the same time as the inner integument does in cottonwood. This integument in very young ovules is only two cells thick at the micropylar end but becomes much thicker in the older ovules.

In both species the ovules are nearly straight when the integuments are initiated (figs. 8, 9). Soon thereafter a curvature appears in the funiculus. Bending continues so that at about the time the megaspore mother cell is fully differentiated, the ovule appears essentially hemitropous (figs. 10, 11). The completely anatropous condition is attained, however, before the megagametophyte has become two-nucleate.



FIGS. 1–21.—Fig. 1, *Populus tremuloides*, pistillate flower. Fig. 2, *P. deltoides*, longisection of young pistillate catkin, ×14. Figs. 3–12, *P. deltoides*, development of pistillate flower: figs. 3–8, ×21; figs. 9–12, ×87; fig. 3, young floral primordia; figs. 4–6, origin and development of carpels; figs. 7, 8, longisections of ovary showing origin of ovules; fig. 9, longisection of ovary showing initial stage in bending of ovule; figs. 10–12, and 15, stages in development of integuments and vascular strand in funiculus. Figs. 13–15, position of megagametophyte, at different stages of development, with respect to nucellus and micropyle. Fig. 16, *P. tremuloides*, staminate flower. Fig. 17, *P. deltoides*, longisection of young staminate catkin, ×14. Figs. 18–21, *P. deltoides*, development of staminate flower, ×21: fig. 18, young floral primordia; figs. 19–21 origin and development of stamens.

A provascular trace differentiates in the funiculus of the ovule of both species at about the time the megaspore mother cell is clearly distinguishable from the wall tissue (fig. 11); this trace begins to mature during tetrad formation (fig. 12). In the mature ovule a distinct vascular strand, four or five cells thick, extends to the chalazal region (fig. 15) but does not enter the integument.

During the four-nucleate megagametophyte stage, rarely earlier, nuclei of the nucellar cells lateral to the gametophyte enlarge, and the cytoplasm increases in amount. By the time the gametophyte is mature, these cells are very conspicuous —much more so in cottonwood, however, than in trembling aspen.

The epidermal hairs which form the cotton of the seed in cottonwood develop on the placentae, not on the ovules, and are initiated at approximately the early eight-nucleate stage of the megagametophyte. Practically every epidermal cell gives rise to a hair. The hairs begin to enlarge at about the time the megagametophyte is fully organized and develop so rapidly that they inclose the ovule completely by the time the zygote undergoes its first division. The hairs are unicellular and uninucleate and remain so. At first each hair has a broad base and a narrow tip and contains a large amount of cytoplasm. The nucleus and a rather conspicuous vacuole appear at the basal end of the hair. As development progresses, both the vacuole and the cytoplasm become less conspicuous, and the nucleus migrates out into the hair. A portion of the placenta bearing these hairs is shed along with the seed. The cotton in trembling aspen appears to be similar to that in cottonwood. There appears to be more in cottonwood, however, probably because of the greater number of ovule-bearing placental lobes.

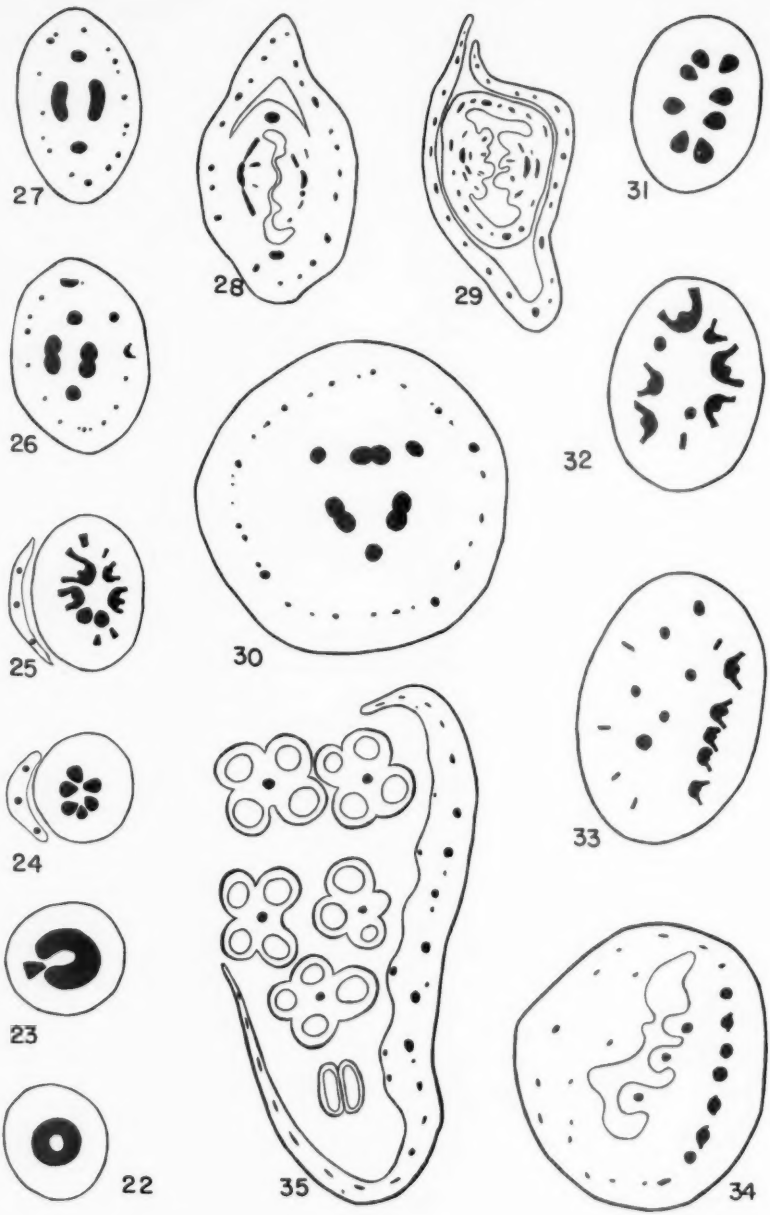
Staminate buds collected in October from both species showed the stamens fairly well outlined. The first stamen primordia develop from the central part of the disk, and from there the development proceeds toward the sides (figs. 19, 20, 21). An examination of several anthers indicates that the staminate buds pass the winter in the microspore-mother-cell stage.

In the pistillate flower of both species typical stomata occur in the outer epidermis of the disk and the ovary. In the staminate flower they are present in the epidermis of the disk and, in *P. deltoides*, in filaments and anthers as well. Stomata were usually found in the region of the connective of the anther and occasionally on the thecae.

VASCULAR ANATOMY OF FLOWER.—In both pistillate and staminate catkins the vascular supply in the peduncle forms a complete cylinder; in the rachis the cylinder is broken by gaps left by traces to the pedicels.

The vascular supply to the pedicel of a two-carpellate pistillate flower (either *P. deltoides* or *P. tremuloides*) passes off from the cylinder of the rachis as a slightly curved segment which soon closes to form a complete cylinder (fig. 22). The bract arising from the pedicel receives a single trace which leaves a single gap (fig. 23). This trace branches into three strands (figs. 24, 25) which pass through the petiole to the base of the blade where each divides one or more times to supply the incised lobes.

After the bract trace is given off, the vascular tissues of the pedicel form a dictyostele (fig. 24). Branches from this pass off (fig. 25) to supply the disk with abundant vascular tissue. The outer and inner tissues of the disk are sharply delimited from each other; the inner receives an abundant vascular supply in



FIGS. 22–35.—Figs. 22–29, *P. tremuloides*, vascular anatomy of pistillate flower; fig. 30, *P. deltoides*, cross section of ovary of tricarpellary flower; figs. 31–35, *P. tremuloides*, vascular anatomy of staminate flower. All ×21.

contrast with the outer, which is entirely destitute of bundles (figs. 27, 28, 29). The major portion of the dictyostele passes into the ovary, where it finally breaks up into four strands—two smaller and two considerably larger bundles (figs. 26, 27). The two smaller ones are the median or dorsal carpellary bundles, one for each carpel; the two larger bundles pass to the placentae. Each of these represents two undiverged ventral carpellary bundles (fig. 27), one from each carpel. Branches from these bundles supply the ovary wall as well as the ovules (figs. 28, 29). Both dorsal and ventral bundles extend into the stigma.

In cottonwood the frequent increase in number of carpels from two to three or four is accompanied, as would be expected, by an increase in number of bundles (fig. 30). Each carpel receives its normal supply of three bundles—one dorsal and two ventral strands. There is at least a partial divergence of the ventral bundles which together comprise the vascular supply to a single placenta. In other respects the vascular supply is essentially the same as in the two-carpellate flower.

The vascular supply to the pedicel and bract of a staminate flower (either *P. deltoides* or *P. tremuloides*) is essentially the same as that of the pistillate flower. After the departure of the bract trace a dictyostele is formed (fig. 31). Branches from this pass off (fig. 32) to supply the disk with abundant vascular tissue. In the disk the branches spread apart and divide and redivide until branches of a third and fourth rank are produced. One fork of each of these, or sometimes the majority of the ultimate subdivisions, becomes the vascular supply to a stamen, while the others supply the periphery of the disk (figs. 33, 34, 35).

MICROSPOROGENESIS.—The stamens are initiated during the early part of the summer of the year preceding the opening of the flower. The young stamen soon becomes distinctly four-lobed in cross section, each lobe representing a microsporangium. In each microsporangial region, as seen in cross section, several large hypodermal cells enlarge radially as archesporial cells and soon divide periclinally to form a layer of primary parietal cells and a layer of primary sporogenous cells (fig. 36). The primary sporogenous cells undergo several successive divisions, forming a group of microspore mother cells, four to sixteen in any one cross section (figs. 37–39). Meanwhile the primary parietal cells undergo periclinal divisions to form usually four, but occasionally five, layers of wall cells (figs. 38, 39).

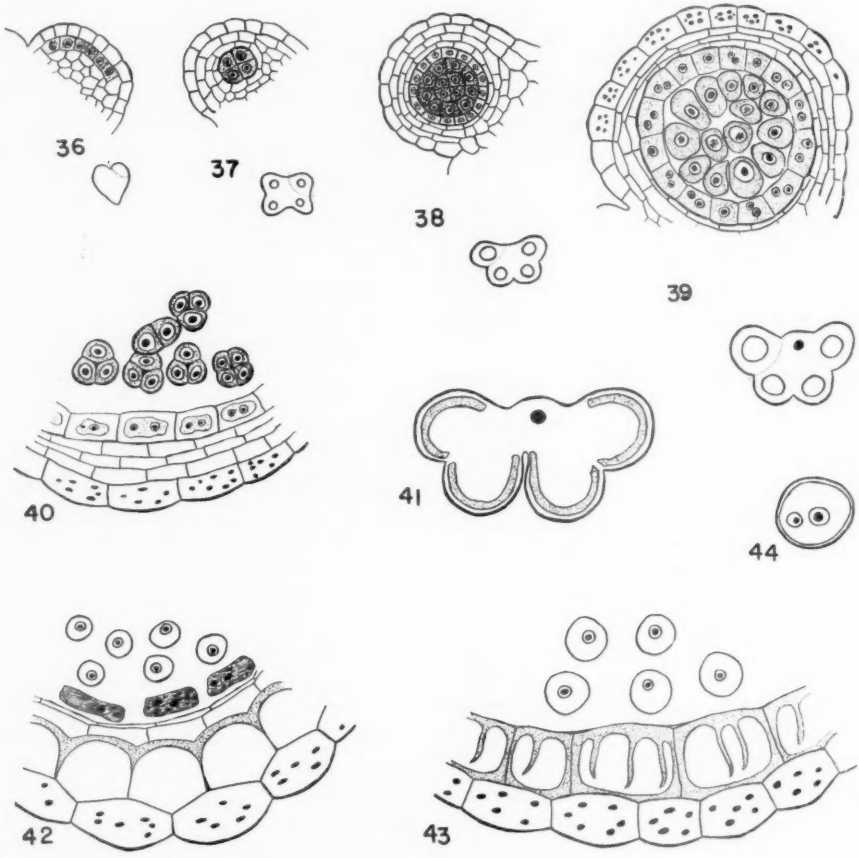
At about the time the microspores separate from one another, the outer wall layer forms an endothecium: thickenings develop on the inner and side walls of the cells as bands extending perpendicularly to the long axis of the anther (figs. 42, 43). Thickenings are found in all cells of the layer except for a few cells between the two anther halves and in the area of actual dehiscence (fig. 41). The inner wall layer matures as a tapetum which is characterized by large, isodiametric cells which are densely granular and binucleate at maturity (fig. 39). These cells remain intact until after the meiotic divisions have occurred. They then disintegrate and are absorbed by the developing spores. In later stages all wall layers between the endothecium and the spores disintegrate (fig. 43).

The microspore mother cells undergo meiosis, and the resulting microspores usually show a tetrahedral, but occasionally an isobilateral, arrangement (fig. 40). Within each spore the nucleus divides to form a generative and a tube nucleus. Division into separate cells was not

observed, but no attempt was made to stain especially for this character. This is the condition of pollen at the time of shedding. Each pollen grain is rather smooth-walled, with a moderately thick exine and a thin intine (fig. 44).

POLLEN GERMINATION.—Pollen grains germinated at all three temperatures used, 11°, 20°, and 30° C., percentage of germination being proportional to temperature as was also tube length. No germination was obtained from any pollen which had been stored, even when stored for only 3 days.

MEGASPOROGENESIS.—A hypodermal archesporium is differentiated at the time



FIGS. 36–44.—*Populus deltoides*, anther structure and microsporogenesis, all ×190 unless otherwise stated; fig. 36, cross section of portion of anther showing archesporial cells, archesporial cells dividing to form primary parietal and primary sporogenous cells; figs. 37, 38, stages in development of sporogenous tissue and wall layers; fig. 39, microspore mother cells at beginning of meiosis; fig. 40, tetrads of microspores just prior to disintegration of tapetum; fig. 41, diagram of cross section of nearly mature anther showing areas of endothecial development, ×46; fig. 42, beginning of thickenings in endothecium; fig. 43, fully developed endothecium; fig. 44, pollen grain at time of shedding, ×317.

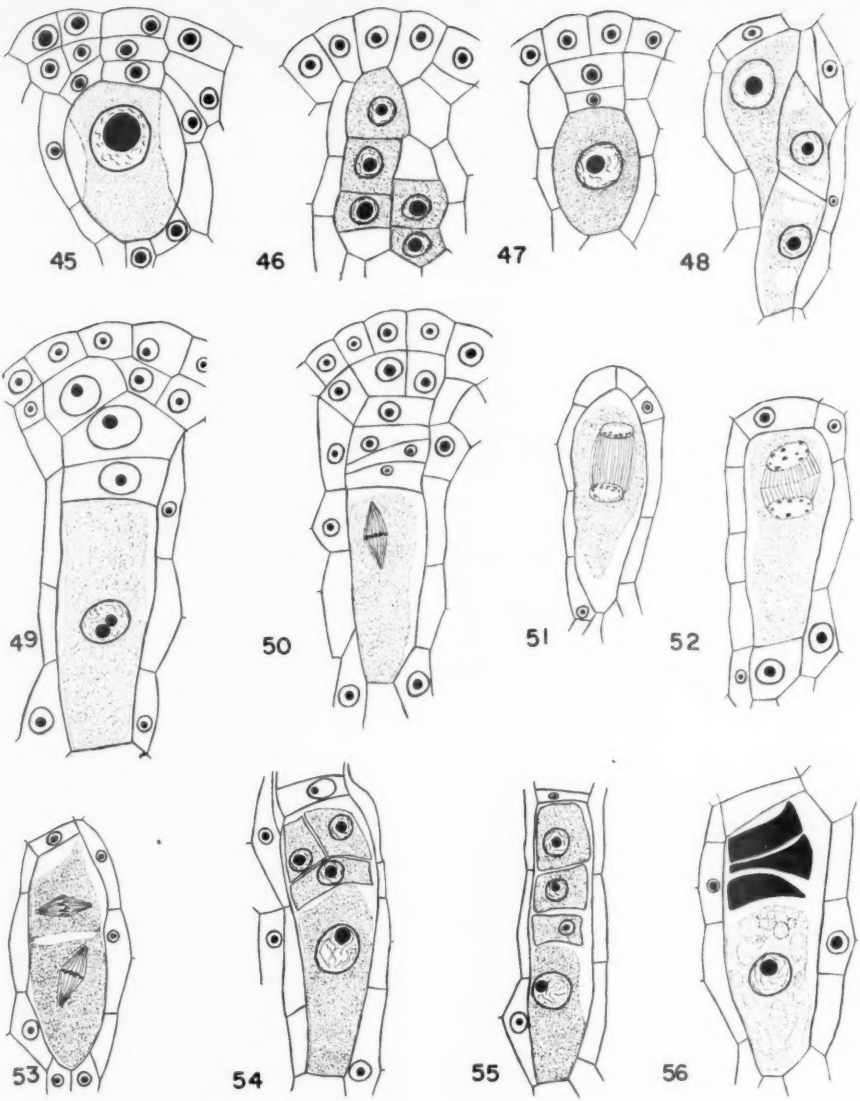
of integument initiation. In cottonwood, although as many as five or six archesporial cells may be formed (fig. 46), usually only one (fig. 47), although occasionally two (fig. 48), and rarely three, will develop. In trembling aspen the archesporium is also hypodermal in origin but is single-celled (fig. 45).

The active archesporial cell divides periclinally to form an outer primary parietal cell and an inner primary sporogenous cell which gives rise, without further division, to the megaspore mother cell. In cottonwood the primary parietal cell and its derivatives undergo further divisions, largely periclinal but occasionally anticlinal, thus forming a layer of three to six cells between the nucellar epidermis and the megaspore mother cell (figs. 49, 50). In trembling aspen, however, the primary parietal cell undergoes only one periclinal division, and the comparable layer consists of two cells only (fig. 45).

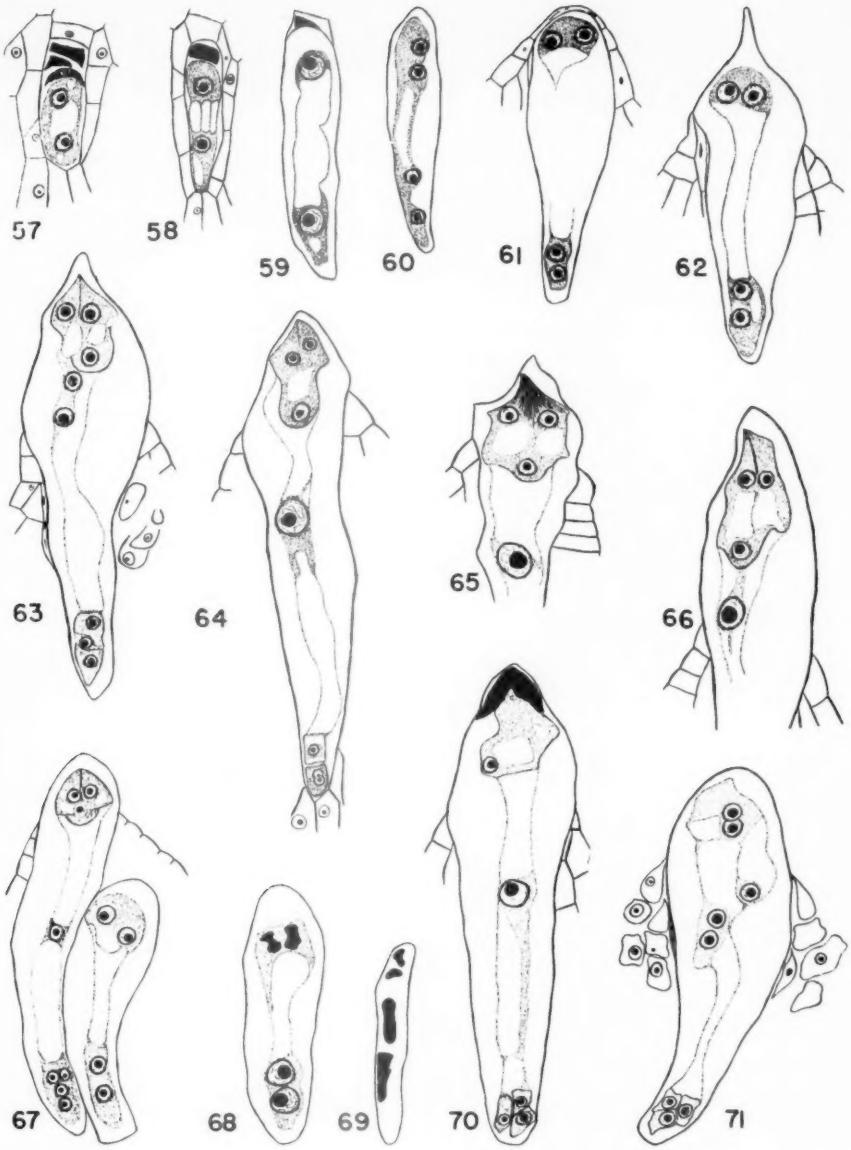
The primary sporogenous cell enlarges considerably to become the megaspore mother cell. The first division of the latter forms a dyad (figs. 48, 50, 51, 52), each cell of which divides again (fig. 53), resulting in formation of the tetrad. A transverse orientation of the second division in the micropylar dyad nucleus usually occurs, and, as the orientation of the chalazal dyad nucleus is longitudinal, a T-shaped tetrad results (fig. 54). Occasionally the orientation of the division figure is longitudinal in both cells of the dyad, resulting in a linear tetrad (fig. 55). Although chromosome counts were not possible, there was nothing in these two divisions to suggest that they were not reductional. The diploid count in *Populus* is reported as 38, the haploid as 19 (5).

DEVELOPMENT OF MEGAGAMETOPHYTE.—Immediately following tetrad formation the three micropylar megaspores begin to degenerate while the chalazal one enlarges (fig. 56). Following the first division of the nucleus of the functional megaspore, a vacuole appears between the two nuclei (fig. 57) and gradually increases in size with further enlargement of the gametophyte (figs. 58, 59). From this stage on the micropylar end of the gametophyte appears to be broad and the chalazal end narrow. The degenerating megaspores normally persist until the two-nucleate stage but usually disappear by the time of the second division which results in the four-nucleate megagametophyte. The two micropylar nuclei of this gametophyte usually lie side by side (fig. 61), but occasionally one lies above the other (fig. 60); the two chalazal nuclei are always one above the other (figs. 60, 61). The early four-nucleate gametophyte is still inclosed by the nucellus (figs. 13, 61), but prior to the third division the gametophyte elongates rapidly, penetrates through the micropylar end of the nucellus, and projects into the micropyle itself (fig. 62). The penetration is apparently accomplished by digestion of nucellar cells by the gametophyte (figs. 63, 71).

The third division gives rise to the eight-nucleate condition, and soon the gametophyte becomes organized (figs. 63–66, 71). During this organization it elongates still more, so that, when completely mature (at the time fertilization would be expected to occur), it has penetrated quite deeply into the micropyle (figs. 14, 15). The egg apparatus is made up of two synergids and the egg. The micropylar end of each synergid is pointed and contains the nucleus, while a large vacuole is located at the chalazal end (fig. 65). In the egg the nucleus lies toward the chalazal end, and there is a vacuole at the micropylar end (fig. 64). The two polar nuclei fuse (figs. 63, 71) to form the



FIGS. 45–56.—All ×708. Fig. 45, *Populus tremuloides*, primary sporogenous cell with two parietal cells. Figs. 46–56, megasporogenesis in *P. deltoides*: fig. 46, multicellular archesporium; fig. 47, primary sporogenous cell with two parietal cells; fig. 48, megaspore mother cell and dyad; fig. 49, primary sporogenous cell with three parietal cells; fig. 50, metaphase of first meiotic division, six parietal cells; fig. 51, 52, telophase of first meiotic division; fig. 53, second meitoic division; fig. 54, T-shaped tetrad; fig. 55, linear tetrad; fig. 56, chalazal megaspore functional with three micropylar megaspores degenerating.



FIGS. 57–71.—All ×354. Figs. 57–70, megagametophyte development in *Populus deltoides:* figs. 57, 58, early two-nucleate megagametophytes; fig. 59, late two-nucleate stage; fig. 60, early four-nucleate stage; fig. 61, four-nucleate megagametophyte just before penetration of nucellus; fig. 62, four-nucleate stage after penetration of nucellus; fig. 63, eight-nucleate stage; fig. 64, mature megagametophyte; figs. 65, 66, micropylar end of mature megagametophyte; figs. 67–70, variations in development of megagametophyte: fig. 67, two megagametophytes in an ovule—one eight-nucleate, one four-nucleate; fig. 68, four-nucleate megagametophyte with two micropylar nuclei degenerated; fig. 69, megagametophyte with all cells degenerated; fig. 70, mature megagametophyte with both synergids degenerated. Fig. 71, *P. tremuloides*, eight-nucleate megagametophyte.

secondary nucleus (figs. 64–66) which is usually found near the egg, although occasionally it may occupy a central position in the gametophyte. In cottonwood the three rather small antipodal cells are usually arranged with one cell at the micropylar end, with the other two side by side, or, occasionally, all are in a linear row at the chalazal end (figs. 63, 64, 70). In trembling aspen the antipodal cells are much larger and are usually arranged with one cell at the chalazal end, the other two side by side (fig. 71).

FERTILIZATION.—In both species fusion of the polar nuclei always precedes the entrance of the pollen tube into the megagametophyte. The pollen tube in cottonwood enters through the micropyle, and, as it penetrates the gametophyte, the synergids usually break down (figs. 72–74). The pollen tube may occasionally enter between the beaks of the synergids. One of the male nuclei fuses with the egg to form the zygote, and the other with the secondary nucleus to form the primary endosperm nucleus (figs. 72, 73). The antipodal cells and any synergid that may not have been destroyed earlier usually disintegrate soon after fertilization.

DEVELOPMENT OF ENDOSPERM.—In both species the development of the endosperm always precedes embryogeny. Endosperm development is of the nuclear type; divisions of the primary endosperm nucleus and its derivatives are not followed by wall formation (figs. 81–83). At first the nuclei lie in a peripheral layer of cytoplasm in the embryo sac (fig. 82), but the central vacuole eventually disappears (fig. 83). Cell-wall formation occurs between the endosperm nuclei at later stages, starting from the periphery and extending inward (fig. 83). The endosperm is completely consumed by the developing embryo so that none remains in the mature seed.

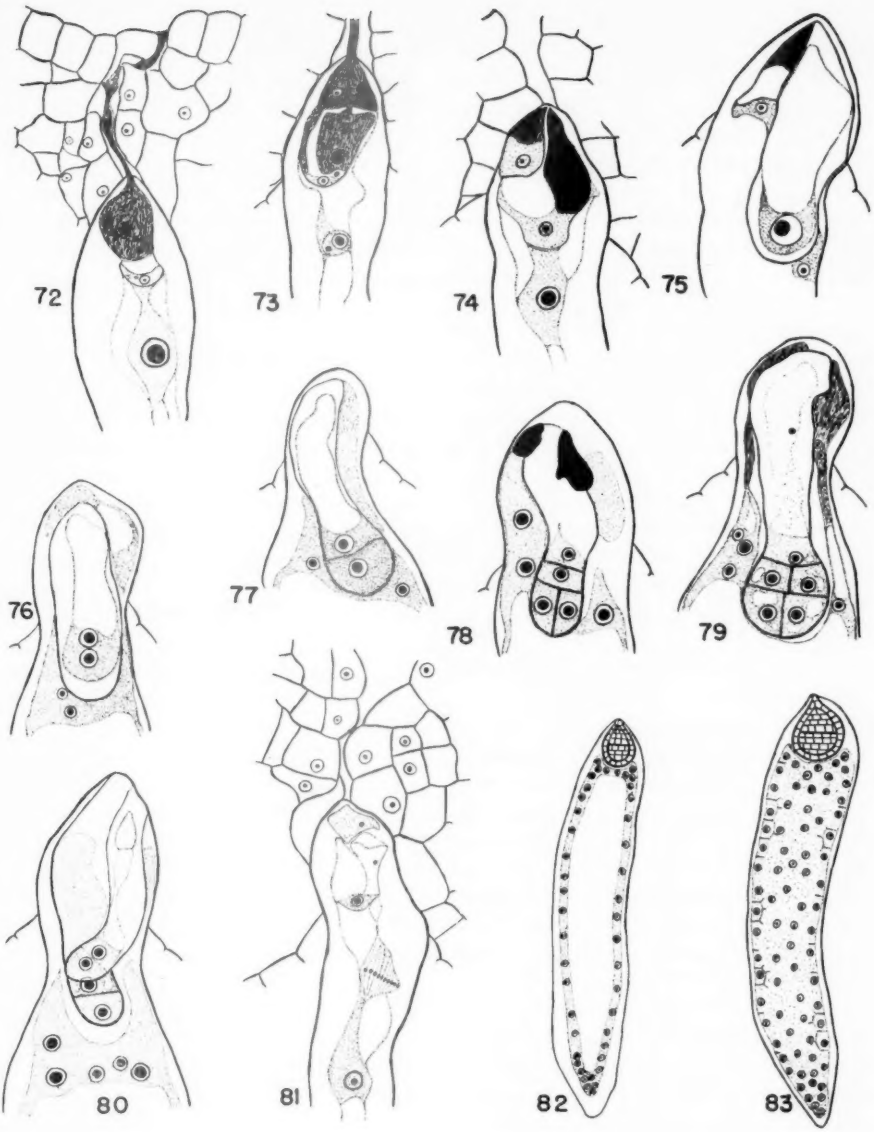
EMBRYOGENY.—In cottonwood the zygote elongates considerably before it undergoes division (fig. 75). The first division of the zygote is always transverse to the long axis of the embryo sac (figs. 76, 77). Occasionally this division separates the zygote into two approximately equal parts, but more frequently the basal cell is larger and somewhat tapering, while its sister cell, which gives rise to the greater part of the embryo, is uniformly hemispherical. The basal cell divides transversely, usually once before the embryo cell divides. The first division of the embryo cell is always longitudinal, and the second division is transverse (figs. 78, 79). The mature embryo is straight with two well-developed cotyledons. The pattern of development of the embryo thus corresponds to the Onagrad Type (**21**). Although detailed studies of embryogeny were not made in the trembling aspen, the few stages observed would indicate that the development resembles that of cottonwood in most respects.

VARIATIONS.—Certain deviations from the typical picture of floral morphology as indicated above have been found in both cottonwood and trembling aspen.

Disintegration of anther locules was rather common in both species but more pronounced in trembling aspen than in cottonwood.

Occasionally two megagametophytes, rarely three, are found in a single cottonwood ovule. In such cases one of them is always at a later stage of development than the other (figs. 48, 67). The appearance of more than one megagametophyte can be accounted for by the development of more than one of the several archesporial cells which are characteristic of this species.

At various stages of megagameto-



FIGS. 72–83.—Fertilization and development of embryo and endosperm. Figs. 72–81, *P. deltoides*, all ×323. Figs. 72–74, fertilization: fig. 72, egg and a male nucleus; fig. 73, secondary nucleus and a male nucleus; fig. 74, one synergid destroyed by pollen tube. Figs. 75–80, embryo development: fig. 75, zygote enlarged before dividing; figs. 76, 77, first division of zygote; fig. 78, first division of embryo cell; fig. 79, second division of embryo cell; fig. 80, two embryos in an ovule. Fig. 81, second division of endosperm. Figs. 82, 83, *P. tremuloides*, ×69. Fig. 82, nuclear endosperm—endosperm nuclei lying in peripheral layer of cytoplasm with vacuole in center; fig. 83, wall formation between nuclei of endosperm, central vacuole disappeared.

phyte development in cottonwood certain nuclei of the gametophyte, rarely all of them, were found to disintegrate (figs. 68–70). For example, certain mature gametophytes were observed in which the synergids had disintegrated, but the other cells were intact and apparently functional, or gametophytes were found in which the antipodals had disintegrated precociously. In trembling aspen the nuclei of the gametophyte at some stages of development, and sometimes all the ovules in a flower, were found to disintegrate.

In an occasional cottonwood ovule where there was no evidence of fertilization, a feebly developed embryo was observed, in some cases with, and in some others without, endosperm. There was no indication that such embryos were derived by proliferation of nucellus or integument, and they may perhaps represent instances of haploid parthenogenesis or apogamy.

One case of polyembryony was observed (fig. 80). One of the two developing embryos was normal, and the other apparently was derived from a synergid.

Occasionally one to three staminate flowers were found toward the base of a pistillate catkin in trembling aspen. These staminate flowers were perfectly normal and produced healthy pollen grains.

Discussion

In the vicinity of the University of Chicago, although staminate catkins of *P. deltoides* usually mature much earlier than pisillate catkins, large amounts of cotton are shed. As suggested in the introduction, there are four possible ways to account for this.

Several observations bear on the nature of the cotton shed. In sections of fruits collected in 1949 and 1950, a very few ovules showed well-developed embryos; most showed evidences of disintegration. Yet the ovary had enlarged and the epidermal hairs which form the cotton were well developed on the placentae. Although shed cotton was not collected and examined, it might be expected that it would have shown either the absence of seeds or the presence of shriveled ones on most of the cotton-bearing pieces of placenta. GRAF (**10**) has suggested the possibility of parthenocarpy in two species of *Populus*. In 1951 cotton was collected but from pistillate trees close to staminate trees which had mature catkins at the time the stigmas were receptive. Most of this cotton showed seeds containing well-developed embryos attached to the pieces of placenta.

HEIZMANN and SHULL (**14**) came to the conclusion that pollination was accomplished by pollen which had been blown around by the wind, or which remained caught in the bark or pistillate bud scales for some time after the staminate catkins fell. Buds of pistillate catkins were examined, but in no case were pollen grains found among bud scales. More important than this, pollen germination tests showed that cottonwood pollen, at least under the variety of conditions used, was viable for only a short period of time. Therefore, HEIZMANN and SHULL's hypothesis cannot be accepted.

During the spring of 1951 observations of cottonwood trees growing in a variety of areas showed many staminate trees to have mature catkins at the time of receptivity of the pistillate flowers. This was true of essentially all trees near Elgin and in many other areas, including some only a few blocks from the University area. It might be possible, therefore, for pollen to be blown in from these locations to the University area, resulting in at least some pollination of the pistillate trees there.

There is also the possibility that seed production may be apomictic. Evidence that all seeds are not apomictic is found in the fact that both staminate and pistillate trees grow from seeds. If all seeds were apomictic in origin, they should perpetuate the parental, that is, pistillate, type only. The apparent lack, or at least deficiency, of pollen during some years does suggest, however, that occasionally seeds may originate by some means other than normal pollination and fertilization.

Apomixis may result (**11, 32**) either from the formation of a diploid gametophyte followed by parthenogenetic development of the egg or apogamous development of some other nucleus of the gametophyte, or from the development of an embryo from a nucellar or integumentary bud. As plants developed from the seeds are apparently normal diploids, the possibility of frequently occurring haploid parthenogenesis is eliminated.

A diploid gametophyte can arise by mitotic division in some cell of the ovule other than the megaspore mother cell or as a result of an upset in the meiotic divisions of the megaspore mother cell. The latter leads to a failure in reduction of the chromosome number so that the functional megaspore is diploid rather than haploid. The embryo in either case must develop parthenogenetically from the egg, apogamously from some other gametophyte nucleus, or by nucellar or integumentary budding.

The reported observations on megasporogenesis in *P. deltoides* eliminate the possibility that the gametophyte develops from a somatic cell. It is the megaspore mother cell which gives rise to the gametophyte, and its divisions therefore become critical. Although it was not possible in any instance to count the chromosomes to determine whether the number was haploid or diploid, there was nothing in any of the division figures to suggest that normal meiosis was not occurring or that the resulting gametophyte was anything but a normal haploid megagametophyte.

There remains, however, the possibility of nucellar or integumentary budding to give rise to the embryo. At no time, from the occurrence of meiotic divisions to the quadrant stage of the embryo, was there any visible differentiation of any integumentary or nucellar cells suggestive of their possible development into an embryo. On the contrary, most embryos observed gave evidence, from their position and general character, of having developed from zygotes. An occasional abnormal embryo, feebly developed, sometimes with, sometimes without, endosperm, may suggest the possibility of occasional incipient haploid parthenogenesis which does not develop far.

From information now available it seems best to conclude that in cottonwood the majority of seeds are produced as a result of normal pollination, followed by fertilization with pollen shed by staminate trees at the time the pistillate catkins are receptive. In cases where pollen must be carried for some distance to effect pollination, not many ovules are fertilized, the nonfertilized ones degenerating. The few which are fertilized, however, are sufficient to result in the maturation of the fruit and formation of cotton on all the placental lobes. All lobes may be shed at maturity whether bearing a well-developed seed or not.

A comparison of the various aspects of the floral morphology of *P. deltoides* and *P. tremuloides* with those of other species of *Populus* and with *Salix* may give some suggestion as to the relative position of *Populus* in the Salicaceae and may also bring some additional evidence to bear

on the question of the relative level of advancement of the genera and the family.

Several aspects of floral morphology have been shown occasionally to be of help in solving taxonomic problems or useful as one line of evidence in determining the relative degree of advancement. Such aspects have been vascular anatomy, staminal structure and certain features of microsporogenesis, ovule structure with megasporogenesis and megagametophyte development, endosperm development, and embryogeny.

In *Populus* each stamen is invariably supplied with a single vascular bundle. Each pistil, consisting of two carpels, receives six bundles—two dorsals and four ventrals. Adjacent ventral strands are usually undiverged so that there are two large placental bundles. The ovary is unilocular with parietal placentae. An exception to the typical bicarpellary condition is found in *P. deltoides*, in which the gynaecium consists of two to four carpels, but usually three. Each of these carpels receives a normal supply of bundles —one dorsal and two ventral bundles, and also they form the typical unilocular ovary. In this plant there is at least a partial divergence of the ventral bundles which together comprise the vascular supply to a single placenta. These observations corroborate those of FISHER (**8**), who has also found similar conditions in both *Salix* and other species of *Populus*. Thus, as far as the vascular anatomy of the flower is concerned, there is fundamentally no difference between the two genera, *Populus* and *Salix*, and the entire flower is simple, whether primitive or reduced, so it is difficult to compare it with the more usual type of flower.

The bracts of both *Populus* and *Salix* are foliar in nature, and the vascular supply to the bract passes off from the vascular cylinder of the pedicel as a single strand leaving a single gap.

Staminate catkins of *Populus* differentiate during the fall of the year prior to opening of the flower. Stamens pass the winter in the microspore-mother-cell stage. MOORE and BEHNEY (**25**), however, have reported mature microspores in buds of *P. deltoides* collected in October, a condition never observed in this species during the present study. The mature anther has a well-developed endothecium, two to three middle layers, and a binucleate tapetum. Microspores are usually tetrahedral in arrangement but occasionally bilateral. Pollen grains are two-nucleate. CHAMBERLAIN's observations of *Salix* (**4**) agree in most respects, except that in *Salix* the number of middle layers may vary from one to four even within a single anther, microspores are always tetrahedral, and pollen grains float in a granular fluid.

Stamen number in *Populus* is relatively high; *P. deltoides* shows sixty or more, but *P. tremuloides* has fewer and sometimes is reduced to only five. Although in *Salix*, section Pleiandrae, there are species with several stamens arranged spirally, in Diandrae and Synandrae the number is reduced to two which in the latter are connate.

In the pistillate buds of *Populus*, which also differentiate during the fall of the year prior to opening of the flower, the ovules remain over winter as conspicuous nucellar primordia, and the integuments do not develop until the following spring. In *P. deltoides* two integuments are initiated in the beginning. The inner integument fuses with the outer just prior to the two-nucleate megagametophyte stage so that at and after this stage the ovule appears to be unitegmic. In *P. tremuloides* there is only one integument. GRAF (**10**) has reported that *P.*

canadensis and *P. canescens* have a weakly developed inner integument in addition to the outer one, while *P. tremula* and *P. alba* have a single integument. The present observation on the two additional species would add further weight to their suggestion that the inner integument is on its way to extinction. A single integument has been reported for all species of *Salix*.

In both genera there are usually several ovules per carpel, but a reduction in number to two per carpel is reported for some species of *Salix: S. heterochroma, S. bracteosa,* and *S. urbaniana,* and a single species of *Populus, P. alba*. In general, however, there are more ovules per carpel in *Populus* than in *Salix*.

The archesporium is hypodermal in origin and consists of several cells in *P. deltoides*, one cell in *P. tremuloides*. A parietal tissue of five or six cells in *P. deltoides* and of two cells in *P. tremuloides* is formed between the nucellar epidermis and the megaspore mother cell. Development of the megagametophyte is of the Normal (*Polygonum*) Type (**22, 23**). The antipodals are ephemeral. The egg apparatus penetrates through the nucellus into the micropyle itself. Several investigators (**4, 10, 12, 24**) have reported a multicellular archesporium and the penetration of the nucellus in many species of *Salix* and in two species of *Populus*. Development of the megagametophyte is of the *Adoxa* or *Allium* Type in *Salix*, according to CHAMBERLAIN (**4**), and of the *Allium* Type in two species of *Populus*, according to GRAF (**10**). MAHESHWARI and ROY (**25**) and HÅKANSSON (**12**) have reported Normal Type development in *Salix*, however. The penetration of the nucellus occurs at an earlier stage in *Populus* than in *Salix*. It is of great interest that HÅKANSSON (**12**) has reported an extreme case in *Salix caprea* where the archesporial cell functions directly as the megaspore mother cell without the formation of parietal tissue.

In *P. deltoides* the pollen tube is porogamous, that is, enters the megagametophyte directly through the micropyle. Both CHAMBERLAIN (**4**) and HÅKANSSON (**12**) have reported similar entry of the pollen tube in *Salix*. GRAF (**10**), however, has reported aporogamy in *P. tremula*, with the pollen tube penetrating the integument of the ovule from the side. It grows inside the integument toward the micropylar end, avoids the micropyle, and continues toward the chalazal end. It finally enters the megagametophyte from the side opposite to that through which penetration of the integument occurred.

As is evident in *P. deltoides* and *P. tremuloides*, the endosperm is of the nuclear type in *Populus* with walls appearing at later stages. Both CHAMBERLAIN (**4**) and HÅKANSSON (**12**) have reported nuclear-type endosperm in *Salix*, but, according to CHAMBERLAIN, the endosperm nuclei are never separated by cell walls.

In *Populus* the polar nuclei fuse to form the secondary nucleus before fertilization. The first division of the zygote is always transverse to the long axis of the embryo sac. The first division of the terminal cell (the embryo proper) is always longitudinal, and the second division is transverse. The development of the embryo thus corresponds to the Onagrad Type (**21**). Early development of the embryo in *Salix* (**4, 31**) is very similar to that of *Populus* and can also be considered of the Onagrad Type.

Although the present study has not conclusively eliminated the possibility of apomixis in *P. deltoides*, where its presence was suspected, there is no good evidence for its occurrence. IKENO (**19, 20**) and BLACKBURN and HARRISON (**2**) have,

however, reported the occurrence of apomixis in two species of *Salix*.

It can be concluded that *Salix* and *Populus* are justifiably placed in a single family. Several aspects, such as a greater number of stamens per flower, the occurrence of bitegmic ovules, a tendency to more archesporial cells, and the development of a heavier parietal tissue, etc., suggest that *Populus* is more primitive than *Salix*, an interpretation which agrees with that of FISHER (**8**). Of the two species of *Populus* studied, *P. tremuloides* may be considered the more advanced. Within the family there has been a tendency in certain characters to change from a condition usually considered more primitive to one more advanced: (*a*) number of stamens reduced from many to two, the two in some species being fused, and reduction accompanied by a change from spiral to cyclic arrangement; (*b*) reduction of carpels from four to two; (*c*) reduction in number of ovules from several to two per carpel; (*d*) change from a bitegmic to unitegmic condition; (*e*) change from an archesporium with many cells to one with only one; and (*f*) change from a well-developed parietal tissue through a reduced amount of it to its complete absence.

Although at one extreme certain of these characters, such as a well-developed vascular strand in the ovule, multicellular archesporium, and considerable parietal tissue, are considered relatively primitive, normally only a single archesporial cell functions (not many as in the Casuarinaceae), and the amount of parietal tissue is reduced in at least one species to the point where it becomes nonexistent. No ovules are reported as showing two well-developed integuments, and most show a single one. A unilocular ovary with parietal placentation, especially when accompanied by reduction in ovule number, may be considered indicative of relative advance. These characters with others, such as consistently anatropous ovules and nonendospermous seeds, would lend support to a more advanced position for the Salicaceae among the angiosperms as given by such classification systems as BESSEY'S (**1**), HALLIER'S (**13**), or HUTCHINSON'S (**18**), rather than an extremely primitive position as in ENGLER and PRANTL'S (**7**) system.

The suggestion that this family with very simple flowers is advanced, implying that the simplicity is a matter of reduction rather than of primitiveness, has been suggested on other bases than the usual taxonomic ones or the ones used here.

HOLDEN'S (**16**) investigation of the details of wood anatomy have indicated that the Salicaceae are of high rank.

FISHER (**8**) has concluded on the basis of extensive morphological studies that (*a*) the simplicity of the flowers is largely the result of extreme reduction and not of the retention of archaic features; (*b*) the flowers once (in ancestral forms) possessed a perianth of certainly one and probably two series which is now represented by the cuplike gland of *Populus* and the usually finger-like gland or glands of *Salix;* and (*c*) the catkins of the Salicaceae represent a very advanced type of inflorescence parallel to the capitulum of the Compositae.

PENHALLOW (**27**) presented the view that the Salicaceae were in process of development during the Tertiary in North America but that in their present condition they are of recent origin. He has also stated that in Cretaceous times the family was compatible with a much warmer climate than at present as evidenced by the presence of the more primitive forms in the tropics.

Summary

1. Various aspects of the floral morphology of *Populus deltoides*, in which a time lag may occur between maturation of the staminate and pistillate catkins, are compared with those of *P. tremuloides*, in which this lag does not occur.

2. Floral buds are differentiated during the fall of the year preceding the opening of the flower. Stamens pass the winter in the spore-mother-cell stage. Nucellar primordia are conspicuous in winter pistillate buds, but the integuments do not appear until spring. In *P. deltoides* the number of carpels varies from two to four, while in *P. tremuloides* it is invariably two. The ovary is unilocular with parietal placentae.

3. The vascular anatomy of the staminate and pistillate flowers is very simple. Each stamen receives a single vascular bundle; each carpel receives the normal supply of vascular bundles—a dorsal and two ventral bundles. The bracts of both staminate and pistillate flowers are foliar in nature, and the vascular supply to the bract invariably passes off from the vascular cylinder in the pedicel as a single strand, leaving a single gap. In both staminate and pistillate flowers the disk is abundantly supplied with vascular tissue. In the pistillate flower, however, only the inner tissue of the disk is supplied with vascular bundles.

4. Microsporogenesis follows the usual angiosperm pattern. The fully developed anther usually shows four layers of wall cells. The outermost consists of an endothecium with fibrous thickenings, and the innermost of a tapetum with cells usually binucleate at maturity. Before the two-nucleate pollen grains are shed, all wall layers between them and the endothecium disintegrate. Stomata occur on the connective and filaments in *P. deltoides*.

5. The ovules are anatropous, and each has a well-developed vascular strand. In *P. deltoides* the ovules are initially bitegmic, but in older ovules only one integument, the outer, can be distinguished. In *P. tremuloides* the ovules are unitegmic.

6. In the ovule archesporial cells are hypodermal in origin. In *P. deltoides* there are five or six, but usually only one develops. It gives rise to a parietal cell and a sporogenous cell. The latter becomes the megaspore mother cell which is rather deep-seated in the nucellus because of several divisions in the parietal cell. In *P. tremuloides* the archesporium is single-celled, and there are only two parietal cells between the nucellar epidermis and the megaspore mother cell.

7. The megaspore mother cell gives rise usually to a T-shaped, or occasionally to a linear, tetrad of megaspores. The chalazal spore is functional and develops into an eight-nucleate megagametophyte (*Polygonum* Type). During the later stages of development the megagametophyte projects through the nucellus into the micropyle.

8. The pollen tube reaches the megagametophyte through the micropyle. Double fertilization occurs. Division of the primary endosperm nucleus precedes that of the zygote and initiates an endosperm of the nuclear type, in which walls appear only at later stages. The first division of the zygote is always transverse to the long axis of the embryo sac. The first division of the embryo cell is always longitudinal, the second division transverse (Onagrad Type).

9. The "cotton" is composed of epidermal hairs of the placentae, not the seeds, and pieces of the placenta are shed with the seeds at maturity.

10. Certain variations of the usual pattern occur. More than one archesporial cell may develop, and thus two or more

megagametophytes may be found in an ovule. Occasional flowers are found in which anther locules, or ovules, are degenerating. Some ovules may show certain or even all cells of the megagametophytes degenerating. Occasionally in *P. tremuloides* one to three staminate flowers occur near the base of the pistillate catkin.

11. Pollen germination tests in *P. deltoides* indicate that pollen grains readily germinate when they are fresh and that they are viable for only a short period of time.

12. The present study has given no clear indication of apomixis in *P. deltoides*, though the possibility has not been conclusively eliminated. Most seeds are produced as a result of pollination by fresh pollen resulting in normal fertilization.

13. The aspects of floral morphology reported correspond very closely to those reported for several species of *Salix* and *Populus* by other workers.

14. The Salicaceae give evidence of being a fairly highly evolved instead of a primitive family and should, therefore, occupy a higher position than that usually accorded to it by the ENGLER and PRANTL system of classification. Within the family *Populus* is more primitive than *Salix*.

The writer wishes to express his appreciation to Dr. B. F. PALSER of the Department of Botany of the University of Chicago for her guidance and encouragement throughout the course of this investigation. He wishes to express his gratitude to other members of the faculty for suggestions and encouragement, and to the Government of Mysore (India) for granting him a Foreign Scholarship without which the present work would have been impossible.

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XYLEM DEVELOPMENT IN YOUNG INTERNODES OF THE KENTUCKY WONDER BEAN

LORA MANGUM SHIELDS AND F. W. SATTLER

Observations were made on plants of *Phaseolus vulgaris* L., the horticultural variety Kentucky Wonder, grown in different photoperiods to determine the relationship between the rate of stem elongation and (*a*) internodal length, (*b*) pith cell length, (*c*) the order of differentiation of the several element types in the earliest maturing xylem, and (*d*) the distances separating individual bands of spiral or annular markings.

Material and methods

Scarred seeds, soaked for 5 minutes in distilled water, were germinated on pads of saturated absorbent cotton in an incubator which was air-conditioned at 32° C. ± 2° and had a relative humidity of approximately 80%. Different rates of stem elongation were induced by growing the bean seedlings for 150 hours in (*a*) continuous light, (*b*) a 13-hour photoperiod, or (*c*) total darkness. The light source consisted of two 14-watt, 14-inch daylight Mazda fluorescent lamps.

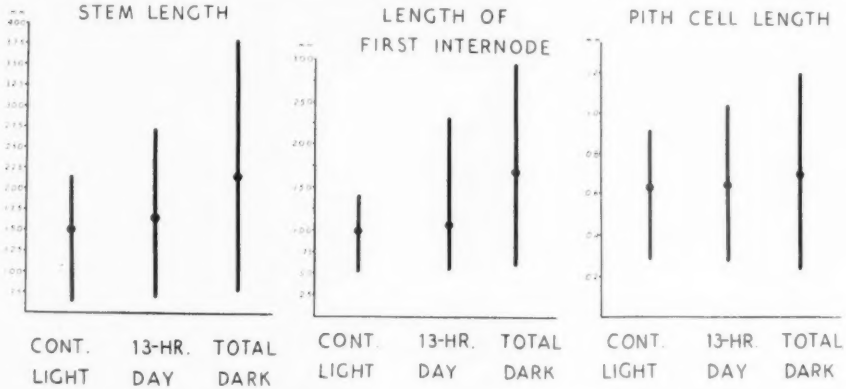
Approximately sixty plants were grown under each of the three conditions of illumination. After the 150-hour growing period measurements were made in millimeters of the stem and internodal lengths of each plant. Entire internodes at higher stem levels and the upper centimeter of all longer internodes were killed and fixed in formalin–acetic alcohol, prepared by the paraffin method, and sectioned longitudinally at 10 μ. Measure-

ments in the stained sections were made under the oil-immersion objective with an ocular micrometer.

Observations

STEM AND INTERNODAL LENGTHS UNDER DIFFERENT CONDITIONS OF ILLUMINATION.—The extent of the inhibitory effect of light on stem elongation is apparent in the maximum and average lengths of stems grown under different daily periods of light exposure (fig. 1). Since all plants in the two groups given illumination produced six internodes and, with twelve exceptions, those grown in complete darkness produced only five, average internodal length was also inversely related to length of daily illumination. The greater height of the 150-hour bean plants grown in total darkness resulted from longer internodes rather than from a greater number of internodes. The exaggerated length of the first differentiated internode in this etiolated group (fig. 2) was largely responsible for the greater height of plants grown in complete darkness.

PITH CELL LENGTH IN RELATION TO STEM ELONGATION.—The average pith cell length in the lowermost internode did not vary significantly with stem length or with the daily period of illumination (fig. 3). A decreased photoperiod apparently results in accelerated division of pith cells proliferating vertically rather than in a stimulation of greater elongation of individual cells. Pith cell length is not necessarily an index to the amount of elongation in adjacent xylem elements, since the meristematic pith cells accommodate to stresses of increasing internodal length by transverse cell division, while early lignified xylem elements become adapted by passive stretching.



FIGS. 1–3.—Effects of daily illumination period on maximum, average, and minimum lengths of (*left*) stem, (*center*) first differentiated internode, and (*right*) pith cells of 150-hour-old plants of *Phaseolus vulgaris* L. var. Kentucky Wonder.

ORDER OF DIFFERENTIATION OF ELEMENT TYPES IN EARLIEST MATURING XYLEM.—It is well known that lignin may be deposited on xylem walls as annular bands, as spirals, as connected spirals or reticula, or, in older areas, as a solid pitted wall. Structurally, the xylem cells formed at any stem level are adapted to the amount of elongation occurring during their formation and functioning period (**2**). Although several observers have found that lignin is deposited in an

annular pattern on walls of the first differentiated xylem elements of different dicotyledonous species (**1–3**), the earliest maturing xylem elements are spirally thickened in the *Medicago sativa* stem (**5**) and show spiral or close reticulate markings in the young *Coleus* shoot (**6**).

In this variety of *P. vulgaris* the isolated, fusiform xylem cells which mature in the first node and second internode below the shoot apex show closely spaced spiral markings. Xylem in the third internode consists of slightly stretched spiral elements. The occurrence of secondary wall markings that can be identified definitely as annular is delayed until the fourth or fifth internode below the stem tip, the levels of most active elongation and pronounced xylary differentiation. These annular markings nearest to the stem apex ordinarily appear as widely separated bands in cells of small diameter on or near the inner face of a bundle including several vertical rows of spirally thickened, and sometimes also reticulate, elements. At this stem level the innermost xylem row is more frequently stretched spiral than annular in plants grown in continuous light, while in etiolated stems annular elements predominate in this position adjacent to the pith. Below the third internode of plants grown in a 13-hour photoperiod, markings on the secondary walls of xylem elements adjacent to the pith are ordinarily annular or spiral in approximately equal numbers. Occasionally scalariform patterns appear on walls of isolated elements of wide diameter adjacent to the pith in sections from plants grown under all three conditions of illumination, possibly because such sections, not being median of the particular bundle at this point, show only a peripheral element. Table 1 summarizes the incidence of the several element types adjacent to the pith in 272 observations made on each of the three groups of plants in the earliest differentiated internode, the fifth or sixth below the stem tip.

Xylem elements having spirally marked walls constitute a larger proportion of protoxylem strands in plants given illumination than in those grown in complete darkness. The increase of total spiral in proportion to annular elements with increasing length of the photoperiod roughly parallels the increasing ratio of spiral to annular elements adjacent to the pith.

TABLE 1

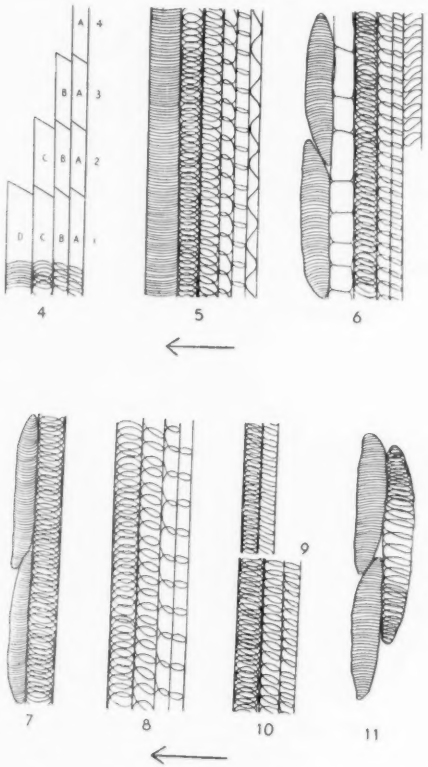
TYPE OF XYLEM ELEMENT ADJACENT TO PITH IN LOWERMOST INTERNODE OF 150-HOUR-OLD PLANTS OF KENTUCKY WONDER BEAN

PHOTOPERIOD	XYLEM ELEMENT TYPE			
	Annular	Spiral	*Vasa mista**	Reticulate
Continuous light	89	141	30	12
13 hours	104	122	33	13
Total darkness	142	82	33	15

* Alternate spiral and annular markings in a single element.

Lignification of secondary xylem walls proceeds simultaneously in many parts of the stem, the type in each element apparently being correlated with longitudinal growth in that element during maturation. In the diagram shown as figure 4, level *1* is the most completely differentiated part of the bundle, and level *4* is the youngest. The order of differentiation assumed at level *1* is *A* first, *B* second, *C* third, and *D* fourth. This interpretation is in agreement with the theory of centrifugal development of the xylem, but the order is not invariably fixed. Element *A* at level *2* may thicken differently from *A* at level *1*, while *A* at *3* may be either spiral or annular, since lignin deposition

occurs independently in each cell. A spiral wall does not give way abruptly to one that is annular but grades into it through alternate spiral and annular markings, designated by GOODWIN (**4**) as *vasa mixta*. Occasionally a stretched spiral element lies next to the pith, while one or two adjacent rows toward the periphery show annular or alternate spiral and annular markings (fig. 5). In general, the first element to become lignified adjoins the pith, but the provascular tissue may mature in such way that certain cells differentiate, while others toward the pith continue to divide, resulting in exceptions to the usual centrifugal succession of element types. Because of such exceptions in the assumed general order of maturation, vessels of larger diameter are occasionally adjacent to the pith (fig. 6). Long strands of provascular tissue sometimes mature while cells lying between them remain parenchymatous (fig. 6).



FIGS. 4–11.—Arrows point in direction of cortex. Fig. 4, usual sequence in differentiation of xylem elements, maturation progressing from 1 to 4 and from *A* to *D*. Fig. 5, stretched spiral element toward pith from annular. Fig. 6, element of larger diameter adjacent to pith; parenchyma separating lignified elements. Fig. 7, lignin markings more closely spaced in narrow than in wider element. Fig. 8, dissimilar patterns of lignification in adjacent elements of equal diameter. Figs. 9, 10, similar spacing of spiral and annular markings in element where two types alternate. Fig. 11, stretched and tight spiral in single element.

Larger element diameter is ordinarily correlated with greater compactness of the lignin deposit on the secondary wall, but markings are sometimes more widely spaced in large than in adjacent narrower elements which apparently have not undergone elongation (fig. 7). Markings on the secondary walls may be dissimilar in two cells of equal diameter lying side by side. Although the approximately equal width of the two cells suggests that secondary wall thickening occurred at about the same stage of development in each (fig. 8), elongation must have accompanied lignin deposition in one and followed it in the other. The closely spaced, spirally marked elements of the first and second internodes of the stem apex, those with both tight and loose spiral markings in the third internode, and the annular, stretched spiral, and close spiral elements below the third node are of essentially the same diameter.

DISTANCES SEPARATING INDIVIDUAL MARKINGS OF SPIRAL AND ANNULAR ELEMENTS IN PLANTS GROWN IN DIFFERENT PHOTOPERIODS.—The lowest, earliest differentiated internode of 150-hour-old *P. vulgaris* plants showed a wider range of

variation in length than other internodes of plants grown under different periods of daily light exposure. Spiral and annular markings in this internode most variable in height were separated by essentially the same distance ranges in corresponding elements of comparable sections from etiolated stems and in those grown under continuous light or on a 13-hour photoperiod. This relative uniformity in the spacing of annular or spiral xylem markings in tall and short plants agrees with the assumed similar lengths of most xylem cells in etiolated stems and in those given continuous illumination, exceptions being a few of the elements differentiated earliest.

It appears that in the earliest-maturing xylem lignin is deposited as spirals in cells where elongation is less rapid during differentiation. When a greater amount of elongation accompanies differentiation, annular deposits are produced, but the distance by which either spirals or annular bands become separated depends upon the amount of stretching after lignification of the secondary wall. Where spiral and annular markings alternate in the same element, the bands are ordinarily separated by no greater distance than the spirals (figs. 8–10). In any individual cell spiral or annular markings tend to remain equally spaced regardless of variations in diameter but are occasionally stretched in some areas and tight in others (fig. 11).

Summary

1. The rate of stem elongation in *Phaseolus vulgaris* L. var. Kentucky Wonder is related to (*a*) internodal length (the inverse relation between stem length and daily period of illumination results from greater elongation of individual internodes in shorter photoperiods rather than from the production of a greater number of internodes); (*b*) pith cell number in a longitudinal direction (etiolation results from vertical proliferation of pith cells rather than from greatly increased length of individual cells); (*c*) the lignification pattern of the earliest differentiated xylem (the ratio of spiral to annular elements adjacent to the pith in the fifth and sixth internodes below the stem apex increases with the daily length of light exposure); and (*d*) the ratio of total spiral to total annular elements (the incidence of spiral markings increases with the length of the photoperiod).

2. The rate of stem elongation has no appreciable effect on (*a*) the total length of individual pith cells or (*b*) the spacing of spiral or annular markings in the first differentiated, longest internode. The distance of separation is determined by the final length of individual cells, which appears to vary little in corresponding positions in etiolated plants, in those given a 13-hour photoperiod, or in those grown in continuous illumination.

3. In the first xylem elements differentiated at the stem tip lignin is deposited as close spirals. In the fourth, fifth, and sixth internodes below the stem apex there are occasional exceptions to a strictly centrifugal pattern of xylem maturation.

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FREE AMINO ACIDS IN SUGAR-BEET LEAVES ALTERED BY ZINC DIMETHYLDITHIOCARBAMATE[1]

ROGER BLOUCH, MERLE G. PAYNE, AND JESS L. FULTS

Introduction

A recent investigation by FULTS *et al.* (**4**) has established that treatment with the fungicide Zerlate results in increases in glutamic acid content of the roots of sugar beet (*Beta vulgaris* L.). Since Zerlate (DuPont 70% zinc dimethyldithiocarbamate) was applied as a foliage spray in the treatment, it seems probable that certain changes in the free amino acid pattern of the leaves may also have taken place. Such alteration, with its consequent effects on normal metabolism, could in part account for the increase in glutamic acid in the root. Since considerable evidence exists of the growth-regulating properties of various carbamates (**2, 3, 5**), it appears entirely possible that significant metabolic changes could have taken place within the entire sugar beet plant.

Investigations on this problem were begun in September, 1950, on sugar-beet plants grown under greenhouse conditions. The results are presented in this report.

[1] This is a contribution of the Botany and Plant Pathology Section and the Chemistry Section of the Colorado Agricultural Experiment Station, Fort Collins, Colorado. Published with the approval of the Director, Colorado Agricultural Experiment Station, as Scientific Series Paper no. 392. Supported in part by a grant from the Herman Frasch Foundation.

Material and methods

Sugar-beet seed of a standard commercial variety was planted in pots on September 25, and the plants were harvested and processed for freezing on November 8. The plants were grown under conditions of regulated moisture and temperature in well-humified, sterilized soil.

The plants were sprayed on three separate dates, October 4, 14, and 24. A stock solution containing 2.5 gm. of 70% Zerlate in 500 ml. of water was applied at the rate of 25 ml. of solution per 1.5 square feet of bench surface area at each application. The acre equivalent for this rate per application is 5.56 pounds per acre. Average plant heights on the above dates were 8, 9.5, and 11 inches, respectively, and average numbers of leaves per plant were six, seven, and eight. Twenty-five treated and twenty-five untreated plants were used in the experiment.

On the harvest date tops and roots were separated and washed. The tops were then arbitrarily subdivided into young, expanding, mature, and old leaves. These units were then weighed, tagged, placed in plastic bags, and frozen.

At time of analysis the samples were

thawed, plant juices extracted, and proteins precipitated with 80% ethanol. The supernatant liquid remaining was drawn off, filtered, and evaporated by a fan to one-fifth of the original volume of juice. In successive trials 5- and 10-microliter (λ) portions were applied to Whatman's no. 1 filter paper for (*a*) total ninhydrin-reacting substances, (*b*) one-dimensional chromatograms, and (*c*) two-dimensional chromatograms for amino acids. The amino acids were identified by the method of DENT (**1**), and relative amounts measured on a Welch Densichron (no. 2150) using a Green N filter.

TABLE 1

EFFECT OF ZINC DIMETHYLDITHIOCARBAMATE ON LEAVES AND ROOTS OF SUGAR BEETS

Values per plant	Treated	Control
Av. no. of leaves	12.1	12.6
Av. length of leaves (cm.)	31.1	36.2
Av. green leaf weight (gm.)	33.4	49.5
Av. fresh root weight (gm.)	4.7	5.9

Results and discussion

Considerable limitation in green weight of leaves and roots occurred with zinc dimethyldithiocarbamate treatment (table 1), indicating that the concentration found suitable in field tests on more mature beets is probably somewhat high for greenhouse application. Differences in numbers and lengths of leaves per plant were small.

Data in table 2 give the statistical analyses of the total ninhydrin-reactants in both roots and leaves. Each value shown is the mean of twenty determinations. Highly significant increases in the roots and in the young and expanding leaves resulted from treatment, but treated mature and old leaves showed no signficant difference from the controls. At this point further work on the roots was discontinued owing to lack of sufficient plant material. However, the root phase had already been intensively investigated in the earlier work (**4**).

TABLE 2

RELATIVE AMOUNTS OF TOTAL NINHYDRIN-REACTING SUBSTANCES IN SUGAR BEETS SPRAYED WITH ZINC DIMETHYLDITHIOCARBAMATE

Type of tissue	Mean Densichron units		Difference	Standard deviation	Standard error	Minimum difference required for significance	
	Treated	Control				0.05	0.01
Young leaves	2.09	1.97	+0.12	0.11	0.02	0.07	0.09
Expanding leaves	1.87	1.74	+ .13	.07	.02	.05	.06
Mature leaves	1.71	1.70	+ .01	.04	.01	.03	.04
Old leaves	1.61	1.59	+ .02	.06	.01	.04	.05
Roots	2.02	1.79	+0.23	0.06	0.01	0.04	0.05

Next, juice samples of young and expanding leaves from both treated and control plants were spotted on one-dimensional chromatograms. These were developed in 80% phenol, air-dried, and sprayed with ethanol containing ninhydrin reagent. (This was made by adding 0.15 gm. of ninhydrin to 50 ml. of 95% ethanol plus two drops of glacial acetic acid.) The amino acids thus identified from treated and control leaves were

compared in concentration by using the Welch Densichron. The mean values obtained were recorded for the analysis shown in table 3. The notable changes included a highly significant increase in glutamine (chief constituent of a spot occupied also by alanine, tyrosine, and methionine sulfone) and a highly significant decrease in glutamic acid (the chief constituent of a spot occupied also by serine). An apparent significant increase in gamma-amino butyric acid (GABA) was found later to be due to the great increase in valine and leucine which have the same approximate Rf as GABA on one-dimensional chromatograms. Aspartic acid showed no significant differences between treated and control leaves.

Two-dimensional chromatograms were then run of concentrated juice samples. These were developed one way in phenol as before, then in lutidine at right angles to the first solvent front. Ninhydrin solution was the test reagent. Fifteen amino acids were identified: aspartic acid, cysteic acid, glutamic acid, serine, asparagine, glycine, threonine, glutamine, alanine, methionine sulfone, tyrosine, gamma-amino butyric acid, valine, leucine or isoleucine, and proline. As in the one-dimensional analysis, glutamine showed increase with treatment and glutamic acid decreased. GABA did not show any differences, but valine and leucine were denser in the treated than in the control samples. Tyrosine, not visible in the controls, was faintly visible in the treated samples.

TABLE 3

FREE AMINO ACIDS IN YOUNG AND EXPANDING SUGAR-BEET LEAVES*

SPOT NO.	AMINO ACIDS	MEAN DENSICHRON UNITS		DIFFERENCE	STANDARD DEVIATION	STANDARD ERROR	MEAN DIFFERENCE REQUIRED FOR SIGNIFICANCE	
		Treated	Control				0.05	0.01
1.....	Aspartic acid Cysteic acid	1.53	1.58	−0.05	0.07	0.02	0.06	0.09
2.....	Glutamic acid Serine	1.68	2.00	− .32	.09	.03	.08	.11
3.....	Glutamine Alanine Tyrosine Methionine sulfone	2.39	2.18	+ .21	.04	.01	.04	.05
4.....	Gamma-amino butyric acid Valine Leucine and isoleucine	2.07	1.99	+0.08	0.03	0.01	0.03	0.04

* These values are based on Welch Densichron readings of one-dimensional chromatograms run in 80% phenol.

Summary

1. The effect of foliage-spray applications of the fungicide Zerlate (zinc dimethyldithiocarbamate) on the free amino acids in sugar-beet leaves grown in a greenhouse was measured in a series of analyses involving total ninhydrin-reactants and a combination of one- and two-dimensional paper chromatography.

2. Glutamic acid content significantly decreased with treatment, and glutamine

significantly increased. Valine and leucine also increased significantly. Tyrosine, undetected in controls, appeared faintly in treated samples.

3. Apparently such treatment with Zerlate induces a metabolic shift wherein glutamic acid decreases in the leaves and accumulates in the roots of sugar beets.

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BLOOMING OF TULIPA CHRYSANTHA AFTER DELAY IN PLANTING[1]

Three to six bulbs of six small species of daffodils and similar quantities of six species of tulips were delayed in transit in the United States postal service from November until the following August. Subsequently they were unpacked and stored dry until the following November, when the surviving bulbs were planted. Three of the original six *Tulipa chrysantha* bulbs had each produced one new offset within the old hulls, and the three others each had two new offsets within the old hulls. Only two bulblets of one other tulip, *Fosteriana cantata,* survived, and each produced two leaves. All the bulbs of the daffodil species survived, but only one plant of *Jonquilla plenus* bloomed. Eight of the nine plants of *T. chrysantha* bloomed, and the plants appeared to be as good as if they had not been delayed a year in planting (fig. 1).

Reproduction without visible growth or root formation has also been observed by the author in three of nineteen bulbs of *T. praestans* which had received secondary irradiation from radioactive bulbs with which they were purposely packed. These bulbs, however, were planted at the normal time and remained in the moist soil until harvest time. The *T. chrysantha* had reproduced within a manila envelope in the cardboard shipping container.

The author would appreciate reports from readers having knowledge of reproduction in bulbs under similar conditions.—TEUNIS VERGEER, *Hope College, Holland, Michigan.*

[1] Finding made incidental to an investigation carried on under a grant from the Joseph Henry Fund, administered by the National Academy of Science.
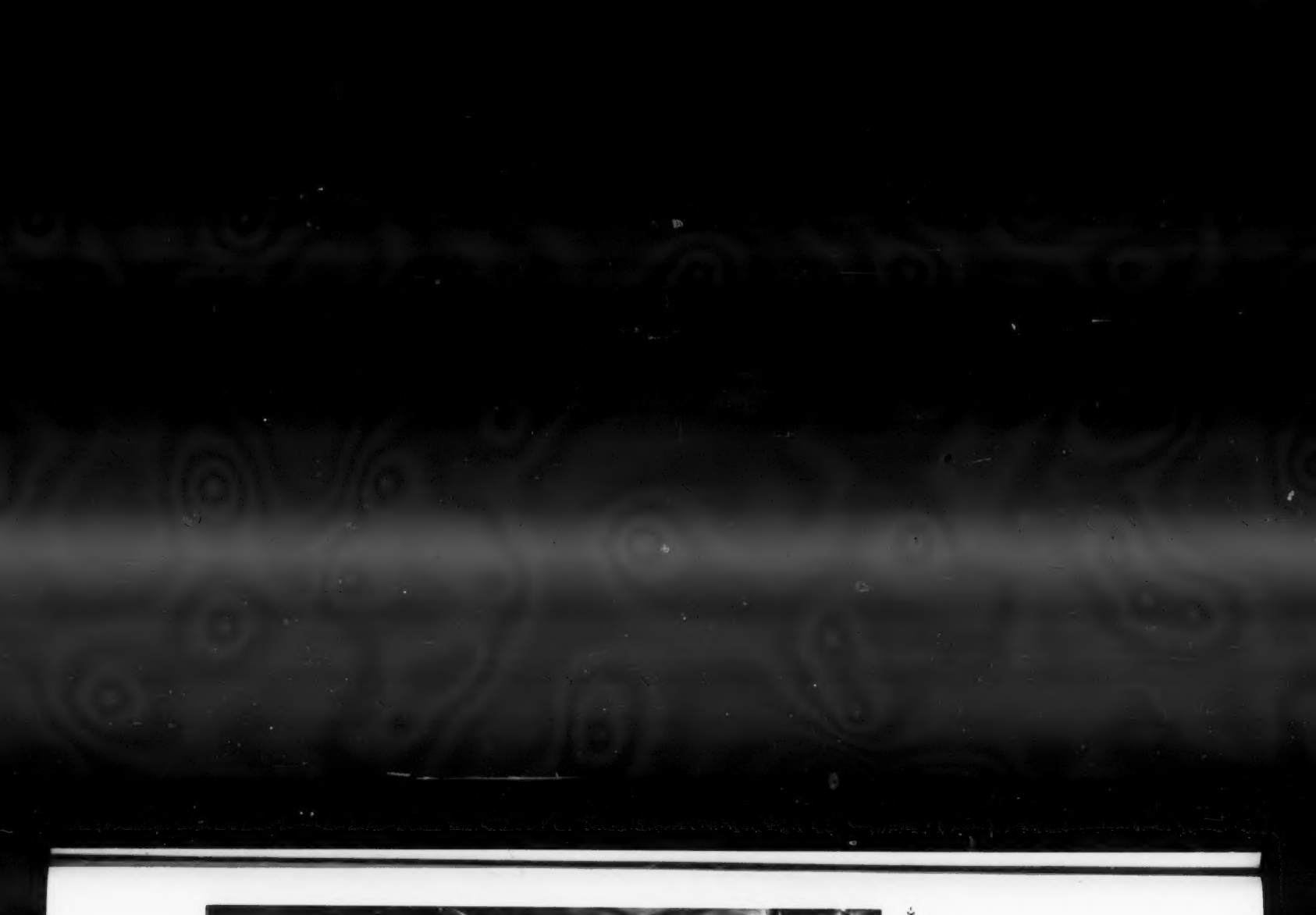
FIG. 1.—*Tulipa chrysantha.* (See text for explanation.)